Consistently High Plasma High-Density Lipoprotein-Cholesterol Levels in Children in Spain, a Country With Low Cardiovascular Mortality

Carmen Garcés, Angel Gil, Mercedes Benavente, Enrique Viturro, Beatriz Cano, and Manuel de Oya

Coronary heart disease (CHD) mortality is relatively low in Spain compared with other developed countries and has remained low despite an apparent increase in mean plasma cholesterol concentration in adults over the last several years. It is accepted that pathologic processes related to arteriosclerosis development begin in childhood and seem to be related to the presence of cardiovascular risk factors at this age. High-density lipoprotein-cholesterol (HDL-C) levels in children have been inversely correlated with the incidence of coronary heart disease in the different countries studied. Childhood plasma lipoprotein profile might contribute to the low coronary heart disease mortality in Spain. Thus, we analyzed data on lipid levels over time in schoolchildren in Spain in the last decade. Plasma lipid levels were analyzed in prepuberal children (6 to 8 years) in 3 school-based surveys performed by our group in Madrid in 1987, 1993, and 1999. A significant increase in plasma total cholesterol ($P < .05$) and low-density lipoprotein-cholesterol (LDL-C) ($P < .01$) levels in prepuberal children was observed over the last decade. However, the mean concentration of plasma HDL-C remained stable and very high. These high levels of plasma HDL-C in Spanish school children may help to explain why the coronary heart disease mortality rate in Spain is low compared with that in other developed countries.

CORONARY HEART disease (CHD) mortality in Spain is much lower than in most developed countries.[1] Furthermore, CHD mortality in Spain has remained low[2] despite a worsening diet and an increase in mean plasma total cholesterol (TC) concentration over the past 3 decades.[3] The apparent inconsistency between increasing TC and low-density lipoprotein-cholesterol (LDL-C) levels and Spain's persistent low CHD mortality remains unexplained.

It is accepted that pathologic processes related to the development of arteriosclerosis begin in childhood and seem to be related to the presence of cardiovascular risk factors at this age. Using intravascular ultrasound, atherosclerotic lesions have been detected in the coronary arteries of 17% of those individuals under 20 studied,[4] indicating the start of the disease at early ages. This suggests that childhood risk factors could be contributing to the incidence of CHD in adults.

A great deal of evidence supports the hypothesis that higher concentrations of either plasma TC or LDL-C increase the risk of CHD, whereas lower concentrations of high-density lipoprotein-cholesterol (HDL-C) increase the risk in adults.[5] Furthermore, the Bogalusa Heart Study[6] and the Pathological Determinants of Atherosclerosis in Youth (PDAY) study[7] have shown that higher concentrations of LDL-C and lower concentrations of HDL-C in children and young people are also associated with a higher risk of atherosclerosis later in life. In general, HDL-C levels in children are inversely correlated with the incidence of CHD in the different countries studied.[8]

We hypothesized that Spain's low CHD mortality could be related to the good lipid profiles of its children. Thus, we analyzed data on lipid levels over time in representative samples of the school age Spanish population from 1987, 1993, and 1999 in Madrid.

SUBJECTS AND METHODS

We analyzed plasma lipid levels in children in the Community of Madrid (Spain) at several different times over the last 15 years. This report makes use of data from 2 of our group's early surveys in the Community of Madrid: the Barrio del Pilar Study of 1987[9] and the Niños de la Comunidad Autónoma de Madrid (NICAM) Study of 1993.[10] Data was also used from the Four Provinces study, a more recent study (1999) that analyzed cardiovascular risk factors in prepuberal children in Spain.[11]

The Barrio del Pilar Study, 1987 Study Sample

This survey[9] was performed with a sample of 476 subjects, of whom 248 children aged 6 to 14 years were included in this current analysis. These children were recruited from several different primary schools in Madrid's Barrio del Pilar neighborhood. For analysis, the children have been categorized according to age in 3 groups: 6 to 8, 9 to 11, and 12 to 14 years old.

NICAM Study Sample 1993

The aim of this study[10] was to find out the lipoprotein distribution in children and adolescents from the Community of Madrid, Spain in 1993. The sample included 3,635 children and adolescents, of which 2,809 between the ages of 6 and 14 were included in this analysis. For this study, children were also categorized by age.

Four Provinces Study 1999

We analyzed a population-based sample of prepuberal children in Spain that included 1,255 healthy schoolchildren between 6 and 8 years old. The study[11] included representative cohorts of schoolchildren from Four Spanish Provinces with different CHD mortalities. Data on Madrid was collected for 324 children in 1999, and these were included in our analysis.

In the 3 studies, participants were free of any endocrine, metabolic, hepatic, or renal disorder, and parents were required to sign a written consent for participation of their children in the study. The study protocols complied with Helsinki Declaration guidelines and Spanish legal provisions governing clinical research on humans and were ap-

From the Lipid Unit, Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Madrid; and the Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos, Madrid, Spain.

Submitted October 3, 2003; accepted March 10, 2004.

Supported by grants from the Fondo de Investigación Sanitaria (FIS 020994, FIS02/3104) and the Ministerio Agricultura Pesca y Alimentación. M.B., E.V., and B.C. are fellows of the Conchita Rábago Foundation.

Address reprint requests to Manuel de Oya, MD, PhD, Unidad de Lípidos, Fundación Jiménez Díaz, Avda. Reyes Católicos, 2, 28040 Madrid, Spain.

0026-0495/04/5308-0025$30.00/0

doi:10.1016/j.metabol.2004.03.012

Table 1. Plasma Lipid Levels for Children in Three Different Studies in 1987, 1993, and 1999 in Madrid

	1987 Study Sample (n = 248)				1993 NICAM Study (n = 2,809)				Four Provinces Study 1999 (n = 324)
	6-8 Years	9-11 Years	12-14 Years	Total	6-8 Years	9-11 Years	12-14 Years	Total	6-8 Years
TC	167.4 (25.7)	177.8 (28.3)	159.5 (22.7)	168.2 (25.5)	176.0 (26.1)	177.8 (26.4)	169.6 (26.9)	174.5 (26.5)	177.9 (27.0)
TG	56.3 (12.1)	58.5 (13.2)	64.8 (12.9)	59.9 (12.7)	53.1 (18.4)	61.3 (20.2)	63.5 (22.3)	59.3 (20.3)	65.9 (23.9)
HDL-C	62.3 (13.4)	63.4 (12.6)	57.2 (11.6)	60.9 (12.5)	62.0 (12.0)	62.6 (13.1)	60.9 (12.9)	61.7 (12.7)	61.1 (12.8)
LDL-C	93.8 (21.7)	101.3 (27.3)	87.6 (19.9)	94.2 (22.9)	103.4 (20.2)	102.9 (19.8)	96.0 (22.3)	100.8 (20.8)	103.9 (23.5)

NOTE. Data are mean ± SD.

proved by the Clinical Research Ethics Committee of the Fundación Jiménez Díaz in Madrid.

Blood sampling, lipid, and apolipoprotein analyses in all 3 studies. Fasting (12-hour) venous blood samples were obtained from every child early in the morning by venipuncture. Plasma cholesterol and triglyceride (TG) levels were determined enzymatically with an RA Autoanalyzer (Technicon, USA). HDL-C was measured after precipitation of apolipoprotein (apo) B-containing lipoproteins with phosphotungstic acid and magnesium (Mg). LDL-C was calculated according to Friedewald's formula.

Statistical Methods

An analysis of variance was used to test the equality of means of independent samples of the 3 studies. For this analysis, children between 6 and 8 years in each study were included. Statistical analyses were performed using the SPSS software package, version 9.0 (SPSS, Chicago, IL).

RESULTS

Table 1 shows the mean concentrations of plasma TC, TG, LDL-C, and HDL-C for the children 6 to 8, 9 to 11, and 12 to 14 in the 1987 Study Sample and in the 1993 NICAM Study. The mean concentrations of those variables in the prepuberal children (6 to 8 years old) included in the Four Provinces Study are also shown. When comparing data between 1987 and 1993, we observed that, for children 6 to 8 and 12 to 14 and for the population considered as a whole, TC and LDL-C levels are increasing, and HDL-C has remained stable.

When analyzing data for the group of prepuberal children (6- to 8-years-old) in the 3 studies, we found a significant increase in plasma TC ($P < .05$) and LDL-C ($P < .01$) levels over the last decade. Levels of TC were 167.4 mg/dL in 1987, 176.0 mg/dL in 1993, and 177.9 mg/dL in 1999. Levels of LDL-C were 93.8 mg/dL in 1987, 103.4 mg/dL in 1993, and 103.9 mg/dL in 1999. However, the mean concentration of plasma HDL-C has remained stable and very high. Mean concentrations of HDL-C were almost identical in the 3 studies (62.3 mg/dL in 1987, 62.0 mg/dL in 1993, and 61.7 mg/dL in 1999). The tendencies of the changes (or lack thereof for HDL-C) were similar in both sexes. The tendencies for mean levels of TC, LDL-C, and HDL-C for this age in each of the 3 studies are displayed in Fig 1.

A total of 19.2% of the child population of Madrid in 1999 had values higher than 200 mg/dL for TC and 12% of the children had values higher than 130 mg/dL for LDL-C. A high percentage of the population, approximately 45%, had higher than 60 mg/dL for HDL-C.

DISCUSSION

While studying changes in plasma cholesterol levels in children in Spain over the last 15 years, we have detected an apparent deterioration of the situation. Over the course of 3 studies performed in Madrid by our group, we detected a significant increase in TC (10.5 mg/dL) and LDL-C (10.1 mg/dL) levels among prepuberal children (6- to 8-year-old children). Nevertheless, along with this increase in TC and LDL-C levels, an analysis of the HDL-C levels in these studies has shown them to be practically unchanged and consistently high between 1987 and 1999. The level of HDL-C in our child population for these years is around 62 mg/dL. All of the studies on school age children performed in Spain[12,13] have found high HDL-C levels. These values are higher than those found in other countries with higher cardiovascular mortality rates than Spain.[8,14,15] Mean levels reported in the United States, for example, were 50.7 mg/dL for Anglo-Americans or 51.3 mg/dL for Latinos.[16] Mahley et al[15] found that, on average, children in Turkey, a country with a relatively high CHD, had 10 to 15 mg less HDL-C than children in other European countries.

It is unknown whether the high HDL-C levels found in our children persist into adulthood and whether they are the cause of our low CHD mortality. There are no important differences in the HDL-C concentrations found in adults from European countries with different CHD mortality rates.[17-19] Even admitting that some of these children may change their lipid levels at

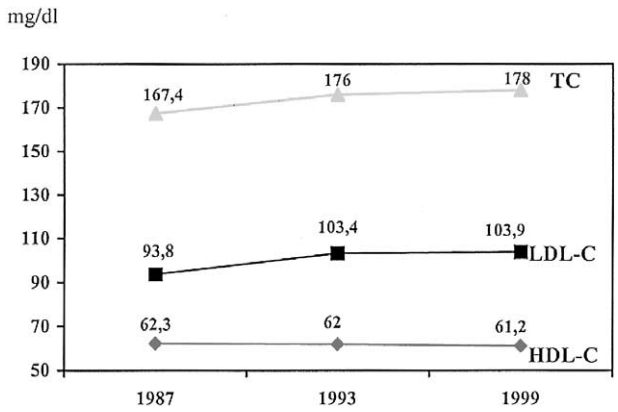

Fig 1. Changes in plasma TC, LDL-C, and HDL-C levels between 1987 and 1999 in prepuberal children (6- to 8-years old) from the Community of Madrid.

puberty and in adulthood, the protective effect that HDL-C is having at this age could be related to the low mortality rates in our population. Today we know that an atherosclerotic lesion begins to form at an early age[4] and the control of high cholesterol levels helps to control the appearance of these lesions and with it the progression of atherosclerosis.

Two possible interpretations for the high and stable levels of HDL-C in our children are (1) that a diet increasingly rich in fats has reached its maximum effect on HDL-C, although LDL-C continues to increase or (2) that the stability of HDL-C has a genetic basis.

When considering the first interpretation (diet), it has been demonstrated that changes in fat intake predict changes in blood cholesterol in Spanish children.[20] In the 1999 Four Provinces Study, the total fat intake for children in Madrid made up 47.3% of their calories.[21] This high consumption of fat could explain the high LDL-C levels, but did not affect HDL-C levels.

The second possibility is that these differences are genetically based. Previous studies have documented the impact of mutations on the ApoE gene on the normal variation of plasma lipid levels in children.[22,23] In the Four Provinces Study, we found that the apoE polymorphisms influence the metabolism of the apo B-containing lipoproteins, but not HDL-C levels.[24,25] Perhaps other genetic determinants in interaction with hormone determinant are more important at this age.

In conclusion, Spanish children, like Spanish adults, have high levels of TC and LDL-C. However, unlike adults, they have high levels of HDL-C. We have seen that HDL-C levels are higher in Spanish children than those found in children from other countries with higher cardiovascular mortality rates. It may well be that these high levels of plasma HDL-C that have remained stable despite an apparent deterioration in our diet explain why the CHD mortality rate in Spain is low compared with that in other developed countries.

ACKNOWLEDGMENT

We thank Alexander G Borun for manuscript revision.

REFERENCES

1. Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, et al: A Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Circulation 90:583-612, 1994

2. Boix R, Aragonés N, Medrano MJ: Tendencias en la mortalidad por cardiopatía isquémica en 50 provincias españolas. Rev Esp de Card 56:850-856, 2003

3. Serra-Majem L, Ribas L, Tresserras R, et al: How could changes in diet explain changes in coronary heart disease mortality in Spain? The Spanish paradox. Am J Clin Nutr 61:1351S-1359S, 1995

4. Tuzcu EM, Kapadia SR, Tutar E, et al: High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults. Evidence from intravascular ultrasound. Circulation 103:2705-2710, 2001

5. Gordon T, Castelli WP, Hjortland MC, et al: High density lipoprotein as a protective factor against coronary heart disease (the Framingham Study). Am J Med 62:707-714, 1977

6. Newman WP, Freedman DS, Voors AW, et al: Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis: The Bogalusa Heart Study. N Engl J Med 314:138-144, 1986

7. PDAY Research Group: Relationship of atherosclerosis in young men to serum lipoprotein cholesterol concentrations and smoking: A preliminary report from the Pathobiological Determinants of Atherosclerosis in Youth Research Group. JAMA 264:3018-3024, 1990

8. Dwyer T, Iwane H, Dean K, et al: Differences in HDL cholesterol concentrations in Japanese, American and Australian Children. Circulation 96:2830-2836, 1997

9. Rubio MJ, Tolosa-Latour M, Bellod P, et al: Lipidemia en relación a la edad. Ann Med Interne 5:435-438, 1988

10. López D, Gil A, Porres A, et al: Perfil lipoproteico en niños y adolescentes de la comunidad autónoma de Madrid. Med Clin 107:366-370, 1996

11. Garcés C, Lasunción MA, Ortega H, et al: Factores metabólicos en población escolar asociados a la mortalidad cardiovascular en los adultos. Estudio Cuatro Provincias. Med Clin (Barc) 118:767-70, 2002

12. Martínez V, Lozano A, Salcedo F, et al: Evolución de las concentraciones plasmáticas de lípidos y lipoproteínas en los escolares entre los años 1992 y 1996: estudio de Cuenca. Med Clin (Barc) 113:736-740, 1999

13. Brotons C, Gabriel R, Muñiz J, et al: Patrón de la distribución de colesterol total y C-HDL en niños y adolescentes españoles: estudio RICARDIN. Med Clin (Barc) 115:644-649, 2000

14. Knuiman JT, Hermus RJJ, Hautvast JGAJ: Serum total and high density lipoprotein (HDL) cholesterol concentrations in rural and urban boys from 16 countries. Atherosclerosis 36:529-537, 1980

15. Mahley RW, Arslan P, Pekcan G, et al: Plasma lipids in Turkish children: Impact of puberty, socioeconomic status, and nutrition on plasma cholesterol and HDL. J Lipid Res 42:1996-2006, 2001

16. Webber LS, Osganian V, Luepker RV, et al: Cardiovascular risk factors among third grade children in four regions of the United States. The CATCH Study. Child and Adolescent Trial for Cardiovascular Health. Am J Epidemiol 141:428-439, 1995

17. Assmann G, Schulte H: Results and conclusions of the prospective cardiovascular Münster (PROCAM) Study, in Assmann G (ed): Lipid Metabolism Disorders and Coronary Heart Disease. Munich, Germany, Medizin Verlag, 1989, pp 87-121

18. Parfitt VJ, Rubba P, Bolton C, et al: A comparison of antioxidant status and free radical peroxidation of plasma lipoproteins in health young persons from Naples and Bristol. Eur Heart J 15:871-876, 1994

19. Sentí M, Masía R, Pena A, et al: Determinantes antropométricos y dietéticos de la concentración sérica de las lipoproteínas de alta densidad en un estudio de base poblacional. Estudio REGICOR. Rev Esp Cardiol 51:979-987, 1998

20. Couch SC, Cross AT, Kida K, et al: Rapid westernization of children's blood cholesterol in 3 countries: Evidence for nutrient-gene interactions? Am J Clin Nutr 72:1266S-1274S, 2000

21. Rodriguez-Artalejo F, Garcés C, Gorgojo L, et al: Dietary patterns among children aged 6-7 years in four Spanish cities with widely differing cardiovascular mortality. Eur J Clin Nutr 56:141-148, 2002

22. Xu CF, Talmud PJ, Angelico F, et al: Apolipoprotein E polymorphism and plasma lipid, lipoprotein, and apolipoprotein levels in Italian children. Genet Epidemiol 8:389-398, 1991

23. Lapinleimu H, Vukari J, Nunikoski H, et al: Impact of gender, apolipoprotein E phenotypes, and diet on serum lipids and lipoproteins in infancy. J Pediatr 131:825-832, 1997

24. Garcés C, Benavente M, Lasunción MA, et al: Gender-specific effects of apo E genotype on plasma lipid levels in a population-based sample of 6-7-year-old children in Spain. Acta Paediatr 91:1039-1043, 2002

25. Garcés C, Benavente M, Ortega H, et al: Influence of birth weight on the Apo E genetic determinants of plasma lipid levels in children. Pediatr Res 52:873-878, 2002