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Reference values for serum lipids and lipoproteins in Spanish adolescents: the AVENA study

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Summary

Objectives: To provide current reference values for serum lipid and lipoprotein levels in Spanish adolescents according to age and sex.
Methods: A cross sectional study conducted in five representative Spanish cities (Granada, Madrid, Murcia, Santander and Zaragoza) including a representative sample of 581 adolescents (299 male and 282 female), aged 13 to 18.5 years. Age- and sex-specific means, standard deviations and percentiles were determined for: Total (TC), high density lipoprotein (HDLc) and low density lipoprotein (LDLc) cholesterol, triglycerides, apolipoprotein A-1 and B-100, and lipoprotein(a).
Results: The 90th percentile for TC was 4.95 mmol/L for males and 5.19 mmol/L for females. HDLc levels were significantly higher in females of all age groups. LDLc levels ranged from 2.32 to 2.54 mmol/L in males and from 2.38 to 2.62 mmol/L in females, peaking at 13 years of age in both sexes. Triglyceride levels tended to increase gradually and to peak at 17 years of age for both sexes. Apolipoprotein A-1 and B-100 levels paralleled those of HDLc and LDLc values, respectively. The geometric mean for lipoprotein(a) levels ranged from 0.44 to 0.57 µmol/L in males and from 0.50 to 0.67 µmol/L in females.
Conclusions: The present study provides reference data on the distribution of lipid and lipoprotein levels of Spanish adolescents.

Keywords: Adolescents – Lipids – Lipoproteins – Cardiovascular disease – Percentiles

Coronary heart disease (CHD) is a leading cause of global mortality, accounting for almost 17 million deaths every year (Smith et al. 2004). Nearly 80 % of this mortality and disease burden occurs in the industrialized countries; the data for Spain reflect this picture (Instituto Nacional de Estadistica, 2001). Pathological data have shown that atherosclerosis begins in childhood (Berenson et al. 1998; Strong et al. 1999), and CHD is known to occur more frequently in adult members of families in which children's cholesterol levels are high. Aortic fatty streaks can be found in children, and fibrous plaques are often evident in adolescence (McGill et al. 1997). This finding, plus the alarming increase in the prevalence of obesity (Moreno et al. 2002; Moreno et al. 2005) and the reduction in physical activity among children and adolescents (Kimm et al. 2002; Moreno et al. 2002; Tercedor 2003), shows the need for improved health education in this age group (Gaziano et al. 1998). The relationship between serum lipids and the development of CHD in children and adolescents is well established (Berenson et al. 1998).
The meta-analysis performed by Plaza (1991) showed that the serum total cholesterol (TC) levels of Spanish children and adolescents increased throughout the 1980s. However, no current data on serum lipid or lipoprotein levels data are available. The AVENA Study was therefore designed to asses the health and nutritional status of a representative population of Spanish adolescents. This report describes the current serum lipid and lipoprotein profiles of Spanish adolescents living in urban areas, and compares the results with those obtained in other countries.

Materials and methods

Population and sample recruitment
The methodology used in this study has been described

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elsewhere (Gonzalez-Gross et al. 2003a, b; Moreno et al. 2005). Briefly, a multicenter study was performed involving a representative sample of Spanish adolescents aged from 13 to 18.5 years. The population was selected by multiple-step, simple random sampling – first taking into account location (Madrid, Murcia, Granada, Santander and Zaragoza) and then by random assignment of the school within each city. The cities were chosen according to the population rate (>100 000 inhabitants), geographical location in the country (north-south gradient, in order to be representative) and taking into account the main technical question, that is, the necessity of having a research group in the city. Sample size was stratified by age and sex. The socio-economic variable was considered to be associated to location within the city and type of school. As the selection of schools was done by random selection proportionally to the number of schools in each city district, guaranteeing the presence of almost one school per district, the socio-economic variable was also considered to be randomly assigned. After analysis of the data, this method has proven to be adequate, as the socio-economic status of our sample has a normal distribution according to the distribution in the Spanish society.

To calculate the number of adolescents to be included in the study in order to guarantee a representative sample of the whole country, we selected the variable with the greatest variance for this age group from the data published in the literature at the time the study was planned; that was body mass index (BMI) (Moreno et al. 1997). The sampling was determined for the distribution of this variable; the CI was established at 95 % with an error ±0.25 %. The minimum subject population was established at 1 750 for the complete study and at 500 for a subgroup from whose member's blood samples were required. A similar number of subjects was evaluated in each city, and proportionally distributed by sex and age group (13, 14, 15, 16, 17–18.5 years).

The sample was oversized in order to prevent loss of information and because technically it was necessary to perform fieldwork in complete classrooms. After finishing the fieldwork, the subjects who did not fulfill the inclusion criteria were excluded. Finally, the sample was adjusted by a weight factor in order to balance the sample in accordance to the distribution of the Spanish population and to guarantee the real representativeness of each of the groups, already defined by the previously mentioned factors (age and sex). The final number of subjects included in the AVENA Study was 2 859 adolescents, from which 581 (299 males and 282 females) had blood measurements, and were then included in this study.

In each school all the adolescents of one classroom were proposed to participate in the survey. A detailed verbal description of the nature and purpose of the study was given to both the children and their teachers. This information was also sent to parents by letter; written consent to be included was requested from both parents and children. The exclusion criteria were: no personal history of cardiovascular or metabolic disease; free of disease and medication at the time of the study; pregnancy. In order to avoid a selection bias, a family history record of metabolic and cardiovascular diseases was obtained for all subjects participating in the study.

The protocol for the complete multicenter study was approved by the Review Committee for Research Involving Human Subjects of the Hospital Universitario Marqués de Valdecilla (Santander, Spain).

Blood measurements

Blood (20 ml) was collected from an antecubital vein between 8:00 and 9:00 a.m, after an overnight fast.

Measurement of serum lipids, lipoproteins and lipoprotein(a)

Total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDLc) were measured by enzymatic assay using a Hitachi 911 Analyzer (Roche Diagnostics, Indianapolis, Ind, USA). HDLc was precipitated before analysis using the Boehringer Mannheim method. Low density lipoprotein cholesterol (LDLc) was calculated using the Friedewald et al. (1972) formula adjusted for serum TG levels (Morley et al. 1998). Apolipoprotein (apo) A-1, apo B-100 and lipoprotein(a) [Lp(a)] were measured by immunonephelometric assay using an Array 306 system (Beckman GMI, Inc., Albertville, Minnesota, USA). Quality control of the assays was assured by the Regional Health Authority. The coefficients of variation were less than 3 % and the intra-class coefficients were higher than 0.96 % for all blood variables. The following atherogenic indices were also calculated: TC/HDLc, TC-HDLc, (TC-HDLc)/HDLc, TG/HDLc, LDLc/HDLc, apo B-100/apo A-1, and apo B-100/LDLc.

Age at menarche was determined from the self-reported date of first menses based on administered questionnaire.

Statistical analysis

For data analysis, the studied population was divided into five age groups: 13–13.99, 14–14.99, 15–15.99, 16–16.99 and 17–18.5 years. Age- and sex-specific means, standard deviations (SD) and percentiles were determined. Kolmogorov-Smirnov test was used to check data distribution by both sex and age and only by sex. The studied variables were quasi-normal distributed, but the asymmetry and kurtosis levels were adequate for all, except for Lp(a) that was achieved after logarithmic transformation. Mean values were compared with one way analysis of variance (ANOVA), and post hoc Bonferroni test. The Mann-Whitney U test was used

to determine any differences in BMI (the variable selected to calculate the number of subjects to be included in the study) between the subgroup from which blood samples were obtained (N = 581) and the remaining subjects (N = 2278) (for each age subgroup and sex). No differences were seen between any of the age and sex groups (Tab. 1). The error was fixed at 0.05.

Results

The means and SDs for lipid and lipoprotein levels, according to age and sex are shown in Table 2. The percentiles distributions for lipid and lipoprotein levels and atherogenic indices, according to age and sex are shown in tables 3–10. Comparison between the sexes shows both higher TC and HDLc levels in females than in males adolescents. Higher LDLc levels were only observed in females aged 15 years ($P < 0.05$). The differences in apo A-1 and apo B-100 levels between the sexes were entirely superimposable on those for HDLc and LDLc levels. Triglycerides levels were slightly lower in females although the differences failed to reach statistical significance, except for the 14 year-olds.

An 8.2 % decline in mean TC serum levels was observed in males between the ages of 13 and 15 years ($P < 0.05$). For males 13 years of age, the 90th percentile for TC (5.39 mmol/L) was the highest estimate for all age groups and both sexes. For females, mean serum TG levels were no different among age groups. For females aged 17–18.5 years, the 90th

Sex	Age group (years)	Body Mass Index		P =
		Blood group (N = 581)	Non Blood group (N = 2278)	
Male	13	20.6 ± 3.3	20.6 ± 4.0	0.63
	14	22.1 ± 3.9	21.4 ± 3.6	0.19
	15	22.3 ± 4.0	21.9 ± 3.5	0.74
	16	21.7 ± 3.3	21.8 ± 3.1	0.86
	17–18.5	23.6 ± 4.2	22.7 ± 3.5	0.15
Female	13	21.0 ± 3.9	21.7 ± 3.6	0.08
	14	21.4 ± 4.2	21.2 ± 3.5	0.35
	15	21.3 ± 3.2	21.5 ± 3.0	0.67
	16	21.9 ± 3.2	21.6 ± 3.1	0.52
	17–18.5	21.8 ± 2.9	21.7 ± 3.3	0.68

Table 1 Comparisons of body mass index between sub-group in which blood sample was obtained (blood group) and group in which blood sample was not obtained (non blood group). Body mass index was calculated as body weight (kg) without shoes and with light clothing, divided by height (m) squared.

Table 2 Lipids and lipoprotein mean and SD values in Spanish adolescents aged 13 to 18.5 years. Values are means ± SD. TC: total cholesterol; HDLc: high density lipoprotein cholesterol; LDLc: low density lipoprotein cholesterol; TG: triglycerides; Apo: apolipoprotein; Lp(a): lipoprotein a. *Geometric mean ± SD. [a]$P < 0.05$ for differences between sexes. [*]$P < 0.05$ (in comparison to males 15 years of age). [¶]$P < 0.05$ (in comparison to males 17 years of age). [#]$P < 0.05$ (in comparison to males 13, 14 and 15 years of age).

Age groups (years)	TC (mmol/L)	HDLc (mmol/L)	LDLc (mmol/L)	TG (mmol/L)	Apo A-1 (g/L)	Apo B (g/L)	Lp(a)[ǂ] (µmol/L)
Males							
13	4.26 ± 0.80[*]	1.35 ± 0.29[a]	2.54 ± 0.66	0.82 ± 0.41	1.16 ± 0.17[a]	0.71 ± 0.18	0.44 ± 0.06
14	4.02 ± 0.59[a]	1.32 ± 0.27[a]	2.32 ± 0.54	0.84 ± 0.41[a]	1.10 ± 0.19	0.67 ± 0.15	0.49 ± 0.05
15	3.91 ± 0.60[a]	1.31 ± 0.23[a¶]	2.24 ± 0.54[a]	0.78 ± 0.28	1.12 ± 0.20[a]	0.65 ± 0.14[a]	0.49 ± 0.04
16	4.07 ± 0.64	1.41 ± 0.27[a]	2.30 ± 0.59	0.79 ± 0.33	1.26 ± 0.20[#]	0.68 ± 0.13	0.48 ± 0.06
17–18.5	4.01 ± 0.73[a]	1.23 ± 0.18[a]	2.39 ± 0.71	0.86 ± 0.37	1.20 ± 0.17[a]	0.70 ± 0.16	0.57 ± 0.06
Total (13–18.5)	4.05 ± 0.68	1.32 ± 0.25[a]	2.35 ± 0.62	0.82 ± 0.36	1.17 ± 0.19	0.68 ± 0.15	0.49 ± 0.05
Females							
13	4.51 ± 0.59	1.53 ± 0.27	2.62 ± 0.52	0.78 ± 0.24	1.24 ± 0.15	0.71 ± 0.11	0.59 ± 0.05
14	4.32 ± 0.70	1.53 ± 0.28	2.48 ± 0.67	0.69 ± 0.27	1.14 ± 0.26	0.72 ± 0.16	0.52 ± 0.05
15	4.38 ± 0.63	1.57 ± 0.33	2.48 ± 0.56	0.72 ± 0.23	1.28 ± 0.24	0.70 ± 0.13	0.55 ± 0.05
16	4.23 ± 0.69	1.53 ± 0.32	2.38 ± 0.58	0.69 ± 0.24	1.29 ± 0.23	0.68 ± 0.13	0.50 ± 0.05
17–18.5	4.40 ± 0.76	1.51 ± 0.28	2.51 ± 0.65	0.83 ± 0.64	1.34 ± 0.22	0.73 ± 0.15	0.67 ± 0.05
Total (13–18.5)	4.37 ± 0.68	1.53 ± 0.30	2.49 ± 0.60	0.74 ± 0.37	1.26 ± 0.23	0.71 ± 0.14	0.56 ± 0.05

Age groups (years)			Total cholesterol				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	4.26	3.32	3.70	4.20	4.69	5.39
14	54	4.02	3.32	3.65	3.94	4.46	4.86
15	63	3.91	3.29	3.56	3.86	4.19	4.68
16	63	4.07	3.34	3.60	4.03	4.43	4.80
17–18.5	65	4.01	3.06	3.46	4.11	4.51	4.95
Total (13–18.5)	299	4.05	3.29	3.57	3.99	4.49	4.95
Females							
13	50	4.51	3.69	4.14	4.45	4.98	5.23
14	55	4.32	3.43	3.78	4.32	4.88	5.23
15	55	4.38	3.68	3.94	4.27	4.78	5.33
16	59	4.23	3.36	3.76	4.12	4.90	5.14
17–18.5	63	4.40	3.51	3.87	4.33	4.94	5.20
Total (13–18.5)	282	4.37	3.52	3.86	4.33	4.87	5.19

Table 3 Mean and percentile distributions for total cholesterol (mmol/L) according to age and sex group. To convert cholesterol values in mmol/L to mg/dL divided by 0.02586.

Age groups (years)			High density lipoprotein cholesterol				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	1.35	0.93	1.11	1.40	1.50	1.68
14	54	1.32	0.91	1.14	1.32	1.53	1.65
15	63	1.31	1.04	1.17	1.32	1.47	1.60
16	63	1.41	1.03	1.24	1.40	1.58	1.79
17–18.5	65	1.23	1.04	1.06	1.22	1.37	1.48
Total (13–18.5)	299	1.32	1.02	1.14	1.32	1.49	1.66
Females							
13	50	1.53	1.16	1.36	1.55	1.68	1.93
14	55	1.53	1.11	1.35	1.50	1.79	1.94
15	55	1.57	1.18	1.37	1.52	1.71	1.99
16	59	1.53	1.09	1.26	1.53	1.72	2.05
17–18.5	63	1.51	1.18	1.30	1.45	1.71	1.90
Total (13–18.5)	282	1.53	1.14	1.32	1.53	1.71	1.95

Table 4 Mean and percentile distributions of high density lipoprotein cholesterol (mmol/L) according to age and sex group. To convert cholesterol values in mmol/L to mg/dL divided by 0.02586.

percentile for TG (1.69 mmol/L) was the highest for all age groups and both sexes. For males, mean LDLc levels tended to decrease gradually from 13 to 15 years of age (11.6%, P = 0.07). In males, the means and percentiles for serum apo A-1 showed distributions similar to those observed for HDLc. In females, apo A-1 levels increased significantly from 14 to 17–18.5 years of age. The percentile distributions for apolipoprotein B-100 were similar to those observed for LDLc, with no differences among age groups for either males or females. Differences were seen, however, between 15 year-old males and females ($P < 0.05$). No gender or age group differences were found in Lp(a) levels. For the atherogenic indices, the TG/HDLc index was significantly higher in males than in females at age 14 and 15 years. Among females, the apo B-100/apo A-1 ratio was significantly higher at 14 compared to 15 years of age.

The self-reported age of menarche ranged from 9 to 15 years of age. The age at first menses distribution was: 9 years (1.6%), 10 years (2.9%), 11 years (20.9%), 12 years (34.7%), 13 years (27.4%), 14 years (11.3%), and 15 years (1%). No differences were observed in serum lipids variables within these groups.

Age groups (years)			Low density lipoprotein cholesterol				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	2.54	1.73	2.07	2.48	2.86	3.39
14	54	2.32	1.67	1.97	2.27	2.61	3.04
15	63	2.24	1.54	1.95	2.19	2.53	2.88
16	63	2.30	1.69	1.92	2.25	2.56	2.98
17–18.5	65	2.39	1.35	1.88	2.37	3.04	3.37
Total (13–18.5)	299	2.35	1.66	1.95	2.31	2.72	3.20
Females							
13	50	2.62	1.87	2.32	2.59	2.94	3.30
14	55	2.48	1.64	1.96	2.45	2.97	3.33
15	55	2.48	1.79	2.09	2.46	2.82	3.27
16	59	2.38	1.67	2.02	2.36	2.72	3.35
17–18.5	63	2.51	1.60	2.02	2.51	2.98	3.27
Total (13–18.5)	282	2.49	1.74	2.07	2.46	2.92	3.30

Table 5 Mean and percentile distributions of low density lipoprotein cholesterol (mmol/L) according to age and sex group. To convert cholesterol values in mmol/L to mg/dL divided by 0.02586.

Age groups (years)			Apolipoprotein A-1				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	1.16	0.95	1.02	1.14	1.29	1.37
14	54	1.10	0.82	0.99	1.13	1.25	1.31
15	63	1.12	0.93	1.02	1.12	1.25	1.37
16	61	1.26	1.02	1.12	1.21	1.40	1.55
17–18.5	57	1.20	0.96	1.06	1.17	1.35	1.43
Total (13–18.5)	290	1.17	0.96	1.04	1.16	1.29	1.42
Females							
13	50	1.24	1.04	1.15	1.24	1.36	1.43
14	55	1.14	0.64	1.06	1.21	1.29	1.42
15	55	1.28	1.02	1.13	1.27	1.46	1.58
16	48	1.29	1.02	1.16	1.27	1.44	1.61
17–18.5	58	1.34	1.10	1.19	1.30	1.45	1.71
Total (13–18.5)	267	1.26	1.02	1.13	1.25	1.38	1.56

Table 6 Mean and percentile distributions for apolipoprotein A-1 (g/L) according to age and sex group. To convert apolipoprotein A-1 values in g/L to mg/dL divided by 0.01.

Discussion

This study provides national reference data for the serum lipid and lipoprotein levels of Spanish adolescents living in urban areas. The percentile distributions according to age and sex are also established. To our knowledge, this is the first report to record the entire serum lipid and lipoprotein profile of a representative sample of Spanish adolescents ranging from 13 to 18 years.

The mean TC, TG and LDLc levels of the present adolescents were similar or slightly lower than those observed in the meta-analysis of Plaza (1991), and then later by Garcés et al. (2004). HDLc levels were also slightly lower than those observed two decades ago (Plaza 1991), perhaps due to a loss of Mediterranean dietary patterns (Moreno et al. 2002; Serra-Majen et al. 1995; Zamora et al. 2003) or to the low level of physical activity recorded for the Spanish population (Moreno et al. 2002) and adolescents of the AVENA study (Tercedor 2003).

According to the NHANES III study, the mean serum TC levels of American children and adolescents aged 12–19 years were 4.09 mmol/L and 4.33 mmol/L for males and females respectively (Hickman et al. 1998). The present Spanish ado-

Age groups (years)			Apolipoprotein B-100				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	0.71	0.49	0.60	0.70	0.79	0.96
14	54	0.67	0.46	0.56	0.64	0.75	0.91
15	63	0.65	0.49	0.57	0.64	0.72	0.87
16	61	0.68	0.51	0.57	0.68	0.75	0.84
17–18.5	57	0.70	0.49	0.60	0.72	0.82	0.91
Total (13–18.5)	290	0.68	0.49	0.58	0.67	0.77	0.88
Females							
13	50	0.71	0.58	0.64	0.71	0.79	0.85
14	55	0.72	0.51	0.60	0.73	0.82	0.93
15	55	0.70	0.52	0.61	0.69	0.78	0.91
16	48	0.68	0.49	0.57	0.69	0.75	0.88
17–18.5	58	0.73	0.54	0.61	0.75	0.83	0.88
Total (13–18.5)	267	0.71	0.53	0.61	0.71	0.80	0.88

Table 7 Mean and percentile distributions for apolipoprotein B-100 (g/L) according to age and sex group. To convert apolipoprotein B values in g/L to mg/dL divided by 0.01.

Age groups (years)			Triglycerides				
	N	Mean	10th	25th	50th	75th	90th
Males							
13	54	0.82	0.36	0.49	0.73	1.11	1.41
14	54	0.84	0.46	0.59	0.70	1.02	1.43
15	63	0.78	0.47	0.57	0.71	0.90	1.16
16	63	0.79	0.42	0.55	0.77	0.94	1.23
17–18.5	65	0.86	0.52	0.64	0.77	1.00	1.36
Total (13–18.5)	299	0.82	0.44	0.58	0.75	0.96	1.31
Females							
13	50	0.78	0.45	0.58	0.76	0.94	1.11
14	55	0.69	0.38	0.50	0.63	0.83	1.00
15	55	0.72	0.45	0.57	0.68	0.86	1.16
16	59	0.69	0.47	0.53	0.62	0.82	0.94
17–18.5	63	0.83	0.40	0.51	0.68	0.81	1.69
Total (13–18.5)	282	0.74	0.44	0.54	0.68	0.84	1.09

Table 8 Mean and percentile distributions for triglycerides (mmol/L) according to age and sex group. To convert triglycerides values in mmol/L to mg/dL divided by 0.01125.

lescents (both males and females) show TC levels similar to those of their American counterparts. The age and sex specific trends for TC levels recorded in the present study were also similar to those reported in the NHANES III study (Hickman et al. 1998). Compared with data from Greece (Shulpis & Karikas 1998), another Mediterranean country, the Spanish mean serum TC levels were slightly higher. The NHANES III (Hickman et al. 1998) and LRC study (Kwiterovich 1991) reported higher TC levels in females than in males; this was also found in the present study. The NHANES III and LRC prevalence studies showed lower TC levels among males during puberty as a result of a decrease in HDLc levels (Hickman et al. 1998; Kwiterovich 1991). This agrees with that seen in the AVENA study. This reduction probably stems from hormonal changes experienced by males during puberty (Kwiterovich 1991). In the present adolescents, the HDLc levels were higher than those recorded for American adolescents (Hickman et al. 1998). This might be attributable to genetic factors, environmental factors and/or to the consumption of olive oil, a major component of the Mediterranean diet (Serra-Majem et al. 1993a, b; Moreno et al. 2002). Therefore, despite having a TC similar to that of American adolescents, the higher HDLc

Age groups (years)			Lipoprotein (a)				
	N	Mean[û]	10th	25th	50th	75th	90th
Males							
13	54	0.44	0.04	0.17	0.54	1.46	3.19
14	54	0.49	0.04	0.21	0.54	1.59	2.83
15	61	0.49	0.07	0.21	0.44	1.02	2.55
16	51	0.48	0.05	0.14	0.40	1.70	3.58
17–18.5	63	0.57	0.10	0.27	0.55	2.45	3.25
Total (13–18.5)	284	0.49	0.05	0.21	0.48	1.50	3.00
Females							
13	50	0.59	0.08	0.28	0.80	1.58	3.71
14	55	0.52	0.05	0.23	0.61	1.74	2.91
15	55	0.55	0.07	0.18	0.49	1.37	3.35
16	55	0.50	0.07	0.21	0.37	1.14	3.00
17–18.5	63	0.67	0.04	0.22	0.57	1.38	3.55
Total (13–18.5)	279	0.56	0.07	0.22	0.55	1.37	3.06

Table 9 Mean and percentile distributions for lipoprotein (a) (µmol/L) according to age and sex group. To convert lipoprotein (a) values in µmol/L to md/dL divided by 0.0357. *Geometric mean.

Table 10 Atherogenic indices in Spanish adolescents aged 13 to 18 years. TC: total cholesterol; HDLc: high density lipoprotein cholesterol TG: triglycerides; LDLc: low density lipoprotein cholesterol; Apo: apolipoprotein. [a]P < 0.05 for differences between sexes. *P < 0.05 (in comparison to girls 15 years of age).

Age groups (Years)	TC/HDLc	TC-HDLc	(TC-HDLc)/ HDLc	TG/HDLc	LDLc/HDLc	Apo B-100 / Apo A-1	Apo B-100 / LDLc
Males							
13	3.15	2.91	2.15	0.60	1.88	0.61	0.28
14	3.04	2.70	2.04	0.63[a]	1.75	0.61	0.29
15	2.98	2.60	1.98	0.59[a]	1.71	0.58	0.29
16	2.90	2.66	1.90	0.56[a]	1.64	0.54	0.29[a]
17–18.5	3.26	2.78	2.26	0.70	1.94	0.59	0.29
Total (13–18.5)	3.06	2.73	2.06	0.62	1.78	0.58	0.29
Females							
13	2.94	2.98	1.94	0.51	1.71	0.57	0.27
14	2.82	2.79	1.82	0.45	1.61*	0.63	0.29
15	2.79	2.81	1.79	0.46	1.58	0.55	0.28
16	2.76	2.70	1.76	0.45	1.56	0.52	0.28
17–18.5	2.92	2.90	1.92	0.55	1.67	0.55	0.29
Total (13–18.5)	2.85	2.83	1.85	0.48	1.63	0.56	0.28

levels of the Spanish youngsters may renders them a healthier lipid profile. Their HDLc levels were, however, lower than those observed in Greek male adolescents aged 13 and 14 years (Shulpis & Karikas 1998). The HDLc levels recorded for females aged 13 and 14 years in the present study were the same as those of the Greek schoolchildren (Shulpis & Karikas 1998). The HDLc levels of Spanish females were higher than that of males; which agree with the results reported by other authors (Azizi et al. 2001). HDLc is a protective factor for females; it is estimated that for every 0.0259 mmol/L (1 mg/dL) increase in HDLc, the risk of a CHD event is reduced by at least by 3 % in females, and 2 % in men (Nicklas et al. 1997).

Low density lipoprotein cholesterol is the main carrier of cholesterol in the blood, and this compound plays a pivotal role in atherogenesis. The mean LDLc levels of Spanish adolescents were similar to those reported in American adolescents (Hickman et al. 1998) but much higher than in Greek adolescents (Shulpis & Karikas 1998). In contrast, Spanish adolescents had much lower TG values than those observed in either

American or Greek adolescents. Comparison of the present serum lipid profiles with those obtained in 26 other countries (Brotons et al. 1998) showed no apparent differences. Since levels of physical activity are rapidly decreasing among Spanish adolescents (Moreno et al. 2002; Tercedor 2003) and the Mediterranean diet is losing its identity (Moreno et al. 2002; Serra-Majen et al. 1995; Zamora et al. 2003), increased obesity, and less favourable metabolic profile is expected to result (Moreno et al. 2002; Moreno et al. 2005). Nowadays, fruit and vegetable intake among Spanish children and adolescents is among the lowest in Europe (Yngve et al. 2005), and an increasing trend in fat consumption during the last decade has been observed (Moreno et al. 2000; Moreno et al. 2002). According to the well known relation between dietary fat, serum cholesterol and cardiovascular diseases (Ascherio et al. 1996), a significant increase in incidence and mortality from cardiovascular diseases should have been detected in Spain. However, this expected trend has not been observed in adults. This has been termed the 'Spanish paradox' (Serra-Majem et al. 1995). This paradox most likely stems from the interaction of multiple synergistic and antagonistic risk and protective factors for cardiovascular diseases.

Relatively little has been published on the apolipoprotein profiles of adolescents. It is therefore difficult to compare the results of the present study with those observed in other cross-sectional examinations. Reference values for apolipoproteins in children and adolescents are of interest since they have been established as new atherosclerosis risk factor (Glowinska et al. 2003). According to some authors, the concentrations of apo A-1 and apo B-100 show an even stronger correlation with atheroma development than their equivalent lipoproteins HDLc and LDLc (Gomez et al. 1996). The levels seen in children have been associated with the incidence of coronary heart disease in their parents (Srinivasan & Berenson 1995). As in adults, the distribution of Lp(a) values was highly skewed towards low values. The geometric means obtained for serum Lp(a) were similar to those reported in Spain in the 1990s (Gomez et al. 1996). However, when median Lp(a) serum concentrations are compared according to age and gender, the figures recorded in the present study are much higher than those reported by Gomez et al. (1996). Assessing new risk factors for atherosclerosis in children and adolescents may provide new insights into the mechanism of formation of atheromatous plaques, especially during the early stages when the process is entirely reversible (Libby 2000). In this regard, the reference values for several atherogenic indices has been provide.

The influence of age at the onset of menses on lipid and lipoprotein concentration is not clear. Associations between age at first menses and TG has been observed (Morrison et al. 1979), whereas either low correlations (Freedman et al. 1987) or no effect of age at first menses has been recently reported (Remsberg et al. 2005). Significant differences in lipid variables according to age of menarche were not observed in this study. This observation was consistent with the above mentioned studies.

The AVENA study included 2 859 adolescents, from which 581 had blood sample. The total number of adolescents to be included in the study was calculated taking into account the variance for BMI (Moreno et al. 1997), as mentioned above. Differences between BMI in the subgroup from which blood samples were obtained and the remaining subjects were not significant (Tab. 1). This suggests that the subgroup with blood data is representative of the whole population.

In conclusion, the serum lipid profile of Spanish adolescents suggests that special attention should be paid to lipid status in this crucial period of life. The present study provides reference data on the distribution of lipid and lipoprotein levels of Spanish adolescents, this information is crucial for planning interventions and education programs promoting the prevention of cardiovascular disease.

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Conflict of interest

No present or past conflict of interest exists for any of the authors or their institutions.

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Zusammenfassung

Referenzwerte für Serumlipide und Lipoprotein bei spanischen Jugendlichen: Die AVENA Studie

Ziel/Objekt: Bereitstellung aktueller Referenzwerte für Serumlipide und Lipoprotein spanischer Jugendlicher nach Alter und Geschlecht

Methode: Querschnittsanalyse durchgeführt in fünf repräsentativen spanischen Städten (Granada, Madrid, Murcia, Santander und Zaragoza); Studienpopulation von 581 Adoleszenten (299 Jungen und 282 Mädchen) im Alter von 13 bis 18,5 Jahren. Alters- und geschlechtsspezifische Mittelwerte, Standardabweichungen und Perzentile wurden bestimmt für: Gesamt (TC), Lipoprotein mit hoher Dichte (HDLc) und Lipoprotein mit niedriger Dichte (LDLc) Cholesterol, Triglyceride, Apolipoprotein A-1 und B-100 und Lipoprotein (a).

Ergebnisse: Die 90igste Perzentile für TC betrug 4,95 mmol/L in der Gruppe der Jungen und 5,19 mmol/L in der Gruppe der Mädchen. Die HDLc-Spiegel waren in allen Altersgruppen signifikant höher bei den Mädchen. Die LDLc-Werte bewegten sich zwischen 2,32 bis 2,54 mmol/L bei den Jungen und zwischen 2,38 bis 2,62 mmol/L bei den Mädchen und waren am höchsten bei den 13-Jährigen beider Geschlechter. Die Werte für Triglyceride wiesen eine steigende Tendenz auf und waren bei den 17-Jährigen beider Geschlechter am höchsten. Die Apolipoprotein A-1 und B-100- Spiegel entsprachen denen von HDLc und LDLc. Der geometrische Mittelwert für Lipoprotein(a) lag zwischen 0,44 und 0,57 µmol/L bei den Jungen und zwischen 0,50 und 0,67 µmol/L bei den Mädchen.

Fazit: Die AVENA Studie stellt Referenzmaterial von Lipiden und Lipoprotein-Spiegeln spanischer Adoleszenter zur Verfügung.

Resumé

Valeurs de référence pour les lipides et lipoprotéines sériques chez des adolescents espagnols. l'étude AVENA

Objectives: Apporter des valeurs de référence actualisées pour les taux sériques de lipides el lipoprotéines par rapport à l'age et au sex.

Méthodes: Une étude transversale fût réalise en 5 villes représentatives (Granada, Madrid, Murcie, Santander et Saragosse) incluant un échantillon représentatif de 581 adolescents (299 garçons et 282 filles), avec un age de 13 à 18.5 ans. Des moyennes spécifiques pour age et sexe, avec des écarts types et percentiles fûrent calculées pour: cholestérol total (TC), cholestérol des lipoprotéines de haute densité (HDLc), cholestérol des lipoprotéines de basse densité (LDLc), triglycérides, apolipoprotéines A-I et B, et lipoprotéine (a).

Résultats: Le percentile 90 pour TC était 4.95 mmol/L pour les garçons et 5.19 pour les filles. Les taux de HDLc étaient significativement plus élevés chez les filles des différentes groups d'age. Les niveaux de LDLc étaient compris entre 2.32 et 2.54 mmol/L chez les garçons, et entre 2.38 et 2.62 mmol/L chez les filles, avec des valeurs plus élevées à 13 ans dans les deux sexes. Les niveaux de triglycérides montraient une tendance à augmenter progressivement jusqu'à 17 ans dans les deux sexes. Les taux d'apolipoprotéines A-1 et B-100 étaient parallèles à ceux de HDLc et LDLc, respectivement. La moyenne géométrique pour les taux de lipoprotéine (a) était comprise entre 0.44 et 0.57 µmol/L chez les garçons et entre 0.50 et 0.67 µmol/L chez les filles.

Conclusions: Le présente étude apporte des valeurs de référence de la distribution des taux de lipides et lipoprotéines chez des adolescents espagnols.

References

Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer M, Willet WC (1996). Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. Br Med J *313*: 84–90.

Azizi F, Rahmani M, Madjid M, et al. (2001). Serum lipid levels in an Iranian population of children and adolescents: Tehran lipid and glucose study. Eur J Epidemiol *17*: 281–8.

Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults: the Bogalusa Heart Study. N Engl J Med *338*: 1650–6.

Brotons C, Ribera A, Perich RM, et al. (1998). Worldwide distribution of blood lipids and lipoproteins in childhood and adolescence: a review study. Atherosclerosis *139*: 1–9.

Freedman DS, Srinivasan SR, Webber LS, Burke GL, Berenson GS (1987). Black-white differences in serum lipoproteins during sexual maturation: the Bogalusa Heart Study. J Chronic Dis *40*: 309–18.

Friedewald WT, Levy RI, Fredrickson DS (1972). Estimation of the concentration of low-density lipoprotein cholesterol in serum, without use of the preparative ultracentrifuge. Clin Chem *18*: 499–502.

Garcés C, Gil A, Benavente M, Viturro E, Cano B, de Oya MC (2004). Consistently High Plasma High-Density Lipoprotein-Cholesterol Levels in Children in Spain, a Country With Low Cardiovascular Mortality. Metabolism *53*: 1045–7.

Gaziano JM (1998). When should heart disease prevention begin? New Engl J Med *338*: 1690–2.

Glowinska B, Urban M, Koput A, Galar M (2003). New atherosclerosis risk factors in obese, hypertensive and diabetic children and adolescents. Atherosclerosis *167*: 275–86.

Gomez Gerique JA, Porres A, Lopez Martinez D, et al. (1996). Levels of lipoprotein(a) and serum lipids in Spanish children aged from 4 to 18 years. Acta Paediatr *85*: 38–42.

González-Gross M, Castillo MJ, Moreno LA, et al. (2003a). Feeding and assessment of nutritional status of Spanish adolescents (AVENA study). Evaluation of risks and interventional proposal. I. Methodology. Nutr Hosp *18*: 15–28.

González-Gross M, on behalf of the AVENA Group. (2003b). The experience of the AVENA multicenter study: planning and development. Ann Nutr Metab *47*: 350.

Hickman TB, Briefel RR, Carroll MD, et al. (1998). Distributions and trends of serum lipid levels among United States children and adolescents ages 4–19 years: data from the Third National Health and Nutrition Examination Survey. Prev Med *27*: 879–90.

Instituto Nacional de Estadística (2001). Defunciones según las causas de muerte más significativa en España. Available at: www.ine.es

Kimm SYS, Glynn NW, Kriska AM, et al. (2002). Decline in Physical Activity in Black Girls and White Girls during Adolescence. N Engl J Med *347*: 709–15.

Kwiterovich PO (1991). Serum lipid and lipoprotein levels in childhood. Ann NY Acad Sci *623*: 90–107.

Libby P (2000). Changing concepts of atherogenesis. J Intern Med *247*: 349–58.

McGill HC, McMahan CA, Malcom GT, Oalmann MC, Strong JP (1997). Effects of serum lipoproteins and smoking on atherosclerosis in young men and women. The PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. Arterioscler Thromb Vasc Biol *17*: 95–106.

Moreno LA, Fleta J, Mur L, Feja C, Sarría A, Bueno M (1997). Indices of body fat distribution in Spanish children aged 4.0 to 14.9 years. J Pediatr Gastroenterol Nutr *25*: 175–81.

Moreno LA, Mesana MI, Fleta J (2005). Overweight, obesity and body fat composition in Spanish adolescents. The AVENA Study. Ann Nut Metab *49*: 71–6.

Moreno LA, Sarriá A, Lázaro A, Bueno M (2000). Dietary fat intake and body mass index in Spanish children. Am J Clin Nutr *72 (5 supplement)*: 1399S–403S.

Moreno LA, Sarría A, Popkin BM (2002). The nutrition transition in Spain: A European Mediterranean country. Eur J Clin Nutr *56*: 992–1003.

Morley R, Baker BA, Greene LC, Livingstone MB, Harland PS, Lucas A (1998). Dietary fibre, exercise and serum lipids and lipoprotein cholesterols in 12 to 15 year olds. Acta Paediatr *87*: 1230–4.

Morrison JA, Laskarzewski PM, Rauh JL et al. (1979). Lipids, lipoproteins, and sexual maturation during adolescence: the Princeton maturation study. Metabolism *28*: 641–9.

Nicklas B, Kaztel LI, Busby-Whitehead J, Goldberg A (1997). Increases in high-density cholesterol with endurance exercise training are blunted in obese compared with lean men. Metabolism *46*: 556–61.

Plaza Pérez y grupo de expertos de las sociedades españolas de arteriosclerosis, cardiología, pediatría, nutrición y medicina preventiva (1991). Report on the cholesterol levels of Spanish children and adolescents. Rev Esp Cardiol *44*: 567–85.

Remsberg KE, Demerath EW, Schubert CM, Chumlea WC, Sun SS, Siervogel RM (2005). Early menarche and the development of cardiovascular disease risk factors in adolescent girls: the Fels Longitudinal Study. J Clin Endocrinol Metab *90*: 2718–24.

Schulpis K, Karikas GA (1998). Serum cholesterol and triglyceride distribution in 7767 school-aged Greek children. Pediatrics *101*: 861–4.

Serra-Majem L, La Vecchia C, Ribas L et al. (1993a). Changes in diet and mortality from selected cancers in southern Mediterranean countries, 1960 – 1989. Eur J Clin Nutr *47 (Suppl 1)*: S25–S34.

Serra-Majem L, Ribas L, Lloveras G, Salleras L (1993b). Changing patterns of fat consumption in Spain. Eur J Clin Nutr *47 (Suppl 1)*: S13–S20.

Serra-Majem L, Ribas L, Tresserras R, Ngo J, Salleras L (1995). How could changes in diet explain changes in coronary heart disease mortality in Spain? The Spanish paradox. Am J Clin Nutr *61 (Suppl 6)*: 1351S–9S.

Smith SC, Jackson R, Pearson TA (2004). Principles for national and regional guidelines on cardiovascular disease prevention: a scientific statement from the world heart and stroke forum. Circulation *109*: 3112–21.

Srinivasan SR, Berenson GS (1995). Serum apolipoproteins A-I and B as markers of coronary artery disease risk in early life: the Bogalusa Heart Study. Clin Chem *41*: 159–64.

Strong JP, Malcom GT, McMahan CA, et al. (1999). Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA *281*: 727–35.

Tercedor Sánchez P, AVENA group (2003). Physical activity in adolescent as a health biomarker in adulthood [Abstract]. Ann Nutr Metab *47*: 351–2.

Yngve A, Wolf A, Poortvliet E, et al. (2005). Fruit and vegetable intake in a sample of 11-year-old children in 9 European countries: The Pro Children Cross-sectional Survey. Ann Nutr Metab *49*: 236–45.

Zamora S, Garaulet M, Pérez de Heredia F, Pérez-Llamas F, AVENA Group (2003). Food habits of the Spanish adolescents in the 21st Century: Is the Mediterranean diet disappearing? [Abstract]. Ann Nutr Metab *47*: 351.

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