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Sincerely

Valeria Hirschler M.D.

Impact of unhealthy lifestyle behaviors and obesity on CETP among adolescent boys.

Running Title: CETP among adolescent boys.

Type of manuscript: Original Article

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Conflict of interests: None

Abstract

Background: Cholesteryl ester transfer protein (CETP) has been proposed to be associated to high risk of cardiovascular disease. Increased CETP activity was previously reported in obese adults, though it association with lifestyle behaviors has not been assessed in healthy adolescents.

Objective: To determine the association between CETP activity and overweight/obesity, insulin resistance markers, components of the metabolic syndrome and lifestyle behaviors in healthy adolescent boys.

Methods: Data were collected from 164 adolescents from an amateur rugby club. Body mass index (BMI), blood pressure (BP), Tanner stages, lipids, glucose, insulin and CETP activity were measured. Questionnaires for daily intake of breakfast, sweet drinks, milk, and hours of TV watching were completed.

Results: About 26% of the adolescents were obese and 23% overweight. The prevalence of metabolic syndrome was 6.7%. CETP activity was higher in obese than in normal and overweight adolescents (174±35, 141±30, and 149±38%/ml.min, respectively; p<0.001). Univariate correlations showed an inverse association between CETP and HDL-C (r=-0.43;p=0.018) and positive ones with BMI (r=0.38;p=0.007), systolic BP (r=0.20;p<0.01) triglycerides (r=0.40;p=0.001), LDL-C (r=0.46;p<0.001), TV watching>2h/day (r=0.17;p 0.02), and milk intake>3glasses/day (r=0.16;p=0.03). Multivariate analysis showed that triglycerides, LDL-C, HDL-C, TV watching>2h/day, milk intake>3glasses/day and BMI were significant independent predictors for CETP (R^2=0.41).

Conclusions: Unhealthy lifestyle habits such as TV watching more than two hours daily and milk intake above three glasses per day and the increase in BMI were

shown to be closely associated to high CETP activity in apparently healthy adolescent boys, thus increasing their risk to develop cardiovascular disease early as adults.

Key Words: obesity, lipid profile, lifestyle behaviors, CETP, atherosclerosis, adolescent boys.

Introduction

The dramatic increase in the prevalence of childhood overweight and obesity is related to comorbidities such as metabolic syndrome and early atherosclerosis [1,2]. The widespread increase in obesity rates has been too rapid to consider genetic factors as the primary cause, and therefore, changes in young people's lifestyle habits have been the focus of most discussions [3].

Childhood obesity is a major public health problem worldwide. The Center for Disease Control and Prevention (CDC) analyzed results from the National Health and Nutrition Examination Survey (NHANES) for 1999—2006 and showed that 32% of youths aged 12-19 years had a high body mass index (BMI) [4]. Moreover, the prevalence of overweight/obesity among school children in Argentina aged 6 to 14 years was 33% [5], very similar to the alarming and increasing rate of obesity among children in the United States. Of particular concern is the fact that obesity related risk factors, that were traditionally attributed exclusively to adults, are now increasingly observed among children [6].

Cholesteryl ester transfer protein (CETP) is a key protein in reverse cholesterol transport which also modulates lipoprotein composition [7]. CETP mass and activity have been the focus of many recent studies as it is considered a determinant factor for plasma high density lipoprotein-cholesterol (HDL-C) levels [8]. Though its role in atherogenesis has not been clearly defined [9-13], several studies described proatherogenic effects of increased CETP activity in association with hypertriglyceridemia [14] and metabolic syndrome [15].

Regarding obesity, the association with CETP was evidenced by prior studies which reported that not only CETP activity and mass were increased in obese patients in comparison to healthy controls, but also that weight reduction partially normalized the altered CETP levels [16-20]. Nonetheless, to our knowledge, CETP activity and its association with lifestyle behaviors have not been assessed in adolescents. Therefore, the aim of the present study was to determine plasma CETP activity and its association with overweight/obesity, insulin resistance markers, components of the metabolic syndrome and lifestyle behaviors in healthy adolescent boys.

Methods

Study design and participants:

Data were collected cross-sectionally from 164 adolescent boys aged 16.7±1.8 years from an amateur rugby club in the north side of Buenos Aires suburbs in April 2009. Exclusion criteria included: missing BMI and blood pressure information, not being in the fasting state, known diabetes or other chronic diseases, the use of medication that could alter blood pressure, glucose or lipid metabolism, and the informed consent not being signed. Of the 189 adolescents recruited, 4 were missing the BMI, 4 the blood pressure and 17 declined to participate. The remaining 164 children were included. All subjects were examined by the same physician. The study was approved by the Human Rights Committee of Durand Hospital in Buenos Aires. Each parent and subject gave written informed consent after an explanation of the study and before its initiation.

Although Argentina is a Spanish-speaking country, the population differs greatly from what is usually referred to as Hispanic in the USA. About 85% of the population is of European descent (largely Spanish and Italian), with the remainder of mixed European and Native American (12%), or Native American (3%) descent.

Socio-demographic characteristics included age and level of education and the presence or absence of a refrigerator or a dirt floor. Questionnaires for socio-economic status have been described in detail elsewhere [21].

Anthropometric measures, blood pressure, metabolic syndrome and stage of puberty:

Height and weight were measured with subjects wearing light clothing and without shoes. Weight was measured to the nearest 0.1 kg on a medical balance scale. Height was measured to the nearest 0.1 cm. with a wall-mounted stadiometer. Adolescents were classified as normal weight (< 85^{th} percentile), overweight (85^{th} to < 95^{th} percentile), or obese (≥ 95^{th} percentile) according to CDC norms [22]. When participants were older than 18 years, they were classified as normal weight (BMI < 25 kg/m^2), overweight (25 kg/m^2 ≤ BMI < 30 kg/m^2), or obese (BMI ≥30 kg/m^2) according to the adult definition [23].

Waist circumference measurement was taken at the level of the umbilicus and recorded to 0.1cm [24]. A non-elastic flexible tape measure was employed with the subject standing without clothing. Central obesity was defined as waist circumference ≥ 94 cm per International Diabetes Federation (IDF) criteria [25].

Three separate blood pressure measurements were recorded by a trained technician using a random-zero sphygmomanometer after the participant was seated at rest for five minutes. The averages of the last two measurements of systolic and diastolic blood pressures were used [26]. Hypertension was defined per IDF criteria [25].

The IDF definition was used for diagnosis of metabolic syndrome [25]

The physical examination also included determination of the stage of puberty according to Tanner criteria [27].

Lifestyle behaviors:

Questionnaires for lifestyle behaviors were completed by the same pediatrician. The questionnaires had been validated in more than 300 children [28]. Food frequency questionnaires are an acceptable measure of patterns of intake [29].

Participants recorded their daily consumption of vegetables and/or fresh fruits, glasses of milk and sweetened beverages, and hours of TV watching, as 1, 2, 3, 4 or $\geq$ 5 as well as the presence of a TV set in the adolescent's bedrooms, and whether or not breakfast was usually consumed. Standard serving sizes and food models were provided as a reference for intake estimation. The daily lifestyle recommendations are as follows: three cups of milk for adolescents, five portions of fruit and vegetables, TV watching less than two hours, the absence of a TV set in the adolescent's bedroom and breakfast intake [30].

Analytical determinations:

Blood samples were obtained from subjects after a 12-hour overnight fast for measurement of glucose, insulin and lipid levels and CETP activity. Plasma glucose and lipid levels were assayed by standardized techniques (Roche Diagnostics, Mannheim, Germany) in a Hitachi 917 analyzer (Hitachi High Technologies Corp., Tokyo, Japan). Plasma insulin levels were measured by radioimmunoassay (Linco Laboratories, St. Charles, MO, USA). The following equation for HOMA-IR index was used: fasting insulin (µU/ml) x fasting glucose (mmol/l)/22.5) [31].

CETP activity:

CETP activity was determined employing an isotopic endogenous assay [32]. After incubation of serum with a tracer amount of biosynthetically labeled HDL_3, apo B-containing lipoproteins were separated by the selective precipitation method.

Data analysis:

Chi squared test was used to compare proportions. When more than 20% of the cells had expected frequencies < 5, Fisher's exact test was used. Data distribution was assessed using the Shapiro-Wilks test. When comparing two groups with normally distributed data, a student t test was performed. When comparing three groups with normally distributed data, one-way Analysis of Variance was used (Student-Newman-Keuls post-hoc test). When the homogeneity of the variances could not be proven, the non-parametric Kruskal-Wallis test was used with Dunn post-hoc test. Given the near normal distribution of plasma CETP activity, untransformed values were used. The correlations with plasma CETP activity were assessed using Spearman correlation coefficients. Plasma CETP activity was modeled as a binary variable (dichotomized at the median) in primary analyses. Additional analyses were performed modeling plasma CETP activity as a continuous variable. Multiple linear regression analysis was performed using the CETP activity as the dependent variable and Tanner stages, lifestyle behaviors, BMI, HOMA-IR and components of the metabolic syndrome as independent variables. P values < 0.05 were considered significant in the two-tailed situation. Data are presented as mean±standard deviation or median (Q1-Q3), depending on data parametric or non parametric distribution, respectively. Analyses were done using the SPSS (Chicago, IL) statistical software package SPSS version 10.0 ®.

Results

Clinical and metabolic characteristics of the adolescent boys :

In the present study, 164 male adolescents were evaluated. General features are shown in table 1.

The adolescent boys ranged from 14 to 22 years old. All subjects were at pubertal or postpubertal stage. The prevalence of Tanner stages 5, 4 and 3 was 51%, 35% and 14%, respectively.

Eighty four (51.2%) adolescent boys had normal weight, 37 (22.6%) were overweight and 43 (26.2%) obese. The prevalence of metabolic syndrome was 6.7% (13/164). Low HDL-C levels (40.2%) and central obesity (25.6%) were common in the sample, while high triglycerides, impaired fasting glucose and hypertension were less frequent (9.1, 7.3, and 6.1%, respectively). Neither of the adolescents had the five components of the metabolic syndrome nor diabetes. Approximately, 55% of the adolescent boys had at least one risk factor for cardiovascular disease and 25% had two or more risk factors.

Adolescent boys belonged to a middle-low socio-economic class, reflected in the educational backgrounds of the parents, with 49.4% of the mothers and 52.7% of the fathers having only an elementary school education or less. All the families had a refrigerator and none had a dirt floor. Approximately, 58% (94/164) of the adolescents watched TV more than two hours per day and 40% (66/164) had TV sets in their bedrooms. Nearly, 95% (156/164) of the children drank one or more glasses of sweet beverages, 11% (18/164) drank more than three glasses of milk, 30% (49/163) did not eat breakfast, and 93% (152/164) ate less than five servings of fruit and vegetables per day. Ninety one percent of the times the sweet beverages consumed by the adolescent boys were juices made from concentrated powder diluted in water. Of interest was that only 19.5% of the adolescents drank low-fat or skim milk, as recommended for children who are older than 2 years (30).

Analysis of data from adolescent boys according to lifestyle behavior:

Clinical and metabolic characteristics according to lifestyle behaviors were also analyzed. Adolescent boys who watched more than two hours of TV per day were compared to those who watched TV two or less hours daily. Moreover, the same analysis was performed between individuals who drank more than three glasses of milk per day and those who drank three or less glasses of milk daily.

There was a higher prevalence of obesity in adolescents who watched TV more than two hours per day than in the other group (91 *vs*. 78%; p<0.05, respectively). Conversely, individuals who drank more than three glasses of milk per day showed a tendency towards a higher prevalence of high triglyceride (22.2 *vs.* 7.5%; p=0.06) and cholesterol levels (27.8 *vs.* 11.6%; p=0.07) in comparison to the other group. In contrast, markers of insulin resistance and CETP activity were not significantly different between TV watching and milk intake subgroups.

Analysis of data from adolescent boys classified according to the presence of overweight and obesity:

Adolescent boys were also divided by the presence of overweight and obesity. Clinical and metabolic characteristics are presented in Table 2. There was not a significant difference in age between the groups.

Deriving form the criteria of classification, BMI and waist circumference were significantly different in the three groups. Mean values of diastolic and systolic blood pressure were higher in obese adolescents than in normal weight and overweight subjects.

Regarding metabolic parameters, insulin concentration, HOMA-IR, and plasma levels of triglycerides, total cholesterol and LDL-C were significantly higher in obese than in normal weight and overweight adolescent boys. On the other hand, HDL-C concentration was significantly lower in obese than in normal weight adolescents. (Table 2). Furthermore, CETP activity was significantly higher in obese adolescent boys than in both normal weight and overweight subjects (Fig. 1).

Analysis of data from adolescent boys classified according to CETP activity:

Accordingly, mean levels of various characteristics are presented separately for adolescent boys with CETP activity dichotomized at the median (below and at or above the median level) in Table 3. Participants with mean values of plasma CETP activity at or above the median had significantly higher BMI, waist circumference, triglyceride, total cholesterol and LDL-C levels but lower HDL-C compared with those with activity below CETP median. Values of systolic and diastolic blood pressure, glucose, insulin and HOMA-IR were not significantly different between both groups.

There was a tendency towards a higher frequency of TV watching more than two hours per day in the group of adolescent boys with CETP activity at or above the median than in the other group (p=0.08). Furthermore, a significantly higher prevalence of adolescents who drank more than three glasses of milk daily was observed in the group at or above median CETP activity (Table 3).

Correlation analysis showed an inverse association between CETP activity and HDL-C concentration (r=-0.43; p=0.018), but positive ones between CETP and BMI (r=0.38; p=0.007), systolic blood pressure (r=0.20; p<0.01), triglycerides (r=0.40;

p=0.001) and LDL-C (r=0.46; p<0.001) levels, TV watching more than two hours per day (r= 0.17; p 0.02), and milk intake more than three glasses daily (r=0.16; p=0.03). In contrast, no significant correlation was found between CETP activity and glucose and insulin levels, HOMA-IR and Tanner stage.

Multiple linear regression analysis using CETP activity as the dependent variable showed that BMI, triglycerides, LDL-C, HDL-C, TV watching more than two hours per day and milk intake above three glasses daily were significant independent predictors for CETP activity adjusted for Tanner stages, sweet drinks intake, systolic blood pressure and total cholesterol ($R^2 = 0.41$) (Table 4). Stepwise analysis showed that the total variance was explained 20% by triglycerides alone, 29% by triglycerides and LDL-C, and 34% by triglycerides, LDL-C and HDL-C. Finally, when BMI, TV watching more than two hours per day and milk intake above three glasses daily were included, 41% of CETP activity total variance could be attributed to the selected model.

Discussion

The most important contribution of the present study was the identification of unhealthy lifestyle behaviors (TV watching more than two hours and milk intake above three glasses daily) as components of the cluster which could explain approximately half of CETP activity total variance. These findings were supported by the results of both univariate and multivariate analyses. Then, CETP activity was associated with lifestyle behaviors and dyslipidemia (high triglyceride and low HDL-C levels), but not with plasma insulin concentration or HOMA-IR. Therefore, in this group of adolescent boys, CETP activity was related to an impaired lipid metabolism and unhealthy lifestyle habits but not to insulin resistance as it was previously suggested [20]. In agreement with the association described with BMI, higher CETP activity was observed in obese adolescent boys. Similar results were reported in prior case-control studies [19,20].

Additionally, CETP activity also showed direct associations with cardiovascular risk factors, which were, in turn, further amplified in the group of subjects with CETP activity at or above the median. These findings are confirmatory of previously reported data obtained from other populations [33].

The prevalence of overweight and/or obesity in the present cohort of Argentinean adolescent boys (48.8%) was higher than the alarming and increasing rate reported among children in USA [4]. This could be due to the fact that overweight and/or obese adolescents are especially encouraged to practice a sport and therefore the prevalence of overweight and/or obesity in this group of rugby amateurs could be overrepresented.

The prevalence of metabolic syndrome in this study (6.7%) was higher than the one reported in a previous study of Argentinean school children (2.5%) [5], possibly attributed to the fact that this population was older.

The reported intake of healthy foods was low in this survey. Approximately, 30% of the adolescent boys skipped breakfast, which is known to be associated with a decreased nutritional quality of the diets [34]. Moreover, a 57.8% of the adolescents watched TV more than 2 hours and 11% drank more than 3 glasses of milk per day. As revealed by subgroup analyses, both lifestyle behaviors were associated with metabolic abnormalities. Fat milk is known to be rich in saturated fatty acids [35], which are associated to higher levels of cholesterol and increased incidence of cardiovascular disease [36,37]. Thus, the association of milk intake with CETP activity could be related to higher lipid levels rather than to a specific effect of milk. Conversely, the positive association between TV watching more than two hours daily (considered a sedentary lifestyle indicator) and CETP activity agrees with a previous report that showed a decrease in CETP concentration in response to exercise training independently of weight loss in adults [38]. These results suggest a specific influence of inactivity and training on CETP, which deserves further investigation.

On the other hand, when the study population was stratified according to CETP activity dichotomized at the median, an adverse lipid and lipoprotein profile was found in adolescent boys at or above median CETP activity. Overall, these results give support to the notion that high CETP activity has a proatherogenic role in adolescents. Nonetheless, whether CETP is proatherogenic or antiatherogenic is still under debate [9-13]. Some controversy might be explained in function of different assays used for the assessment of CETP activity [9-13]. In the present study, CETP activity was evaluated employing endogenous substrate method. This method, that shows a relatively low association with CETP mass [13], is the one that best reflects the *in vivo* situation in which CETP activity is modulated by endogenous plasma factors beyond the mass itself [39].

Recently, a prospective investigation of a community-based sample showed that individuals with plasma CETP activity (assessed by an exogenous assay) below the median level experienced higher cardiovascular disease incidence [12]. In contrast, Zeller *et al.* [13], using an endogenous assay similar to the one employed in this study, observed that high CETP activity was associated with occurrence of the first myocardial infarction at a younger age. Thus, the controversy between these studies could rely on the different methods employed to measure CETP activity and/or in the differences in the baseline lipoprotein profile of the subjects included in each study. In fact, it was suggested that high CETP activity could be considered proatehrogenic in a frame of hypertriglyceridemia and antiatherogenic in the presence of normal triglyceride levels [9,11,13].

Several limitations of this study should be acknowledged. Firstly, it was a cross-sectional analysis, and thus, the directionality of the associations cannot be established. However, appropriate analysis of cross-sectional data represents a useful initial step when identifying relations between BMI, lifestyle behaviors and lipid profile with CETP activity. Secondly, fasting insulin and HOMA-IR were used as surrogate markers of insulin resistance, instead of the gold standard of the hyperinsulinemic euglycemic clamp. Lastly, the use of self-reported food-frequency questionnaires could be also considered a limitation. Nevertheless, the large sample size and the high level of significance of the results are more likely to confirm these findings.

In conclusion, unhealthy lifestyle habits such as TV watching more than two hours daily and milk intake above three glasses per day and the increase in BMI were shown to be closely associated to high CETP activity in apparently healthy adolescent boys, thus increasing their risk to develop cardiovascular disease early as adults. Future longitudinal studies should be done to further confirm these findings.

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Figure Legends

Figure 1. CETP activity from normal weight (n=84), overweight (n=37) and obese (n=43) adolescent boys. CETP cholesteryl ester transfer protein.

[a] p=0.0001 in comparison to normal weight and overweight adolescents.

Table 1. Clinical and metabolic characteristics of adolescent boys.

	Mean/(median)	SD/(Q1-Q3)
n	164	
Age (years)	16.7	1.8
BMI (Kg/m^2)	26	5
Waist (cm)	84	13
DBP (mmHg)	72	9
SBP (mmHg)	116	12
Glucose (mmol/l)	4.5	0.6
Insulin (mU/l)	(6.3)	(4.3-8.3)
HOMA-IR	(1.3)	(0.8-1.8)
TG (mmol/l)	(0.9)	(0.6-1.2)
TC (mmol/l)	4.1	0.9
HDL-C (mmol/l)	1.1	0.3
LDL-C (mmol/l)	2.6	0.9
CETP (%/ml.h)	151	36

SD, standard deviation; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; HOMA, homeostasis model assessment; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein, CETP, cholesteryl ester transfer protein. Data are expressed as mean and standard deviation or median and Q1-Q3, depending on parametric or non parametric distribution, respectively.

Table 2. Clinical and metabolic characteristics of adolescent boys according to the presence of overweight and obesity.

	Normal Weight	Overweight	Obese	Significance
n (%)	84 (51.2)	37 (22.6)	43 (26.2)	
Age (years)	16.6±1.8	16.6±1.9	16.8±1.9	NS
BMI (Kg/m^2)	21.8±2.3	26.2±1.5	32.4±3.1	0.0001[a]
Waist (cm)	75±7	86±7	101±9	0.0001[a]
DBP (mmHg)	71±9	68±7	77±9	0.0001[b]
SBP (mmHg)	114±10	113±10	125±12	0.0001[b]
Glucose (mmol/l)	4.5±0.6	4.4±0.6	4.7±0.6	NS
Insulin (mU/l)	5.2 (3.6-7.1)	6.1 (4.4-7.7)	9.4 (6.4-12.7)	0.0001[b]
HOMA-IR	1.0 (0.7-1.5)	1.2 (0.9-1.6)	2.1 (1.2-2.7)	0.0001[b]
TG (mmol/l)	0.8 (0.6-1.1)	0.8 (0.6-1.1)	1.2 (0.9-1.6)	0,0001[b]
TC (mmol/l)	3.9±0.9	3.8±0.6	4.4±0.9	0.005[b]
HDL-C (mmol/l)	1.2±0.4	1.1±0.3	1.0±0.2	0.001[c]
LDL-C (mmol/l)	2.5±0.9	2.4±0.6	3.1±0.8	0.0001[b]

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; HOMA, homeostasis model assessment; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; NS, non significant. Data are expressed as mean±standard deviation or median (Q1-Q3), depending on parametric or non parametric distribution, respectively.

[a] Significance found between each group.

[b] Significance found comparing obese to normal weight and overweight subjects.

[c] Significance found comparing normal weight to obese subjects.

Table 3. Clinical and metabolic characteristics of adolescent boys according to CETP activity.

	CETP activity (median = 151 %/ml.h)		
	< median	≥ median	Significance
n	82	82	
Age (years)	16.5±1.8	16.9±1.9	NS
BMI (Kg/m^2)	24.4±4.3	26.7±5.5	0.001
Waist (cm)	81±11	88±14	0.001
DBP (mmHg)	71±8	73±10	NS
SBP (mmHg)	115±11	118±12	NS
Glucose (mmol/l)	4.5±0.6	4.6±0.7	NS
Insulin (mU/l)	6.0 (4.2-7.6)	6.5 (4.3-8.9)	NS
HOMA-IR	1.2 (0.8-1.6)	1.3 (0.9-1.9)	NS
TG (mmol/l)	0.8 (0.6-1.0)	1.0 (0.7-1.3)	0.001
TC (mmol/l)	3.8±0.8	4.3±0.9	0.005
HDL-C (mmol/l)	1.2±0.4	1.0±0.2	0.001
LDL-C (mmol/l)	2.4±0.8	2.9±0.9	0.001
TV>2 h [n,(%)]	63 (75.4)	70 (85.7)	0.08
Milk>3 glasses [n,(%)]	3 (3.7)	15 (18.3)	0.002

CETP, cholesteryl ester transfer protein; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; HOMA, homeostasis model assessment; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TV>2h, TV watching more than two hours daily; Milk>3 glasses, milk intake above three glasses per day; NS, non significant. Data are expressed as mean±standard deviation or median (Q1-Q3), depending on parametric or non parametric distribution, respectively.

Table 4: Multiple regression analysis for the association of CETP activity as a dependent variable.

Model	B	Std. Error	t	Significance	R^2
Triglycerides	0.13	0.04	2.75	0.0001	0.41
LDL-C	0.30	0.07	4.07	0.0001	
HDL-C	-0.59	0.19	-3.00	0.0001	
TV>2h	13.75	5.77	2.38	0.02	
Milk>3 glasses	18.90	7.20	2.62	0.01	
BMI	1.08	0.49	2.17	0.03	

CETP, cholesteryl ester transfer protein; LDL, low density lipoprotein; HDL, high density lipoprotein; TV>2h, TV watching more than two hours daily; Milk>3 glasses, milk intake above three glasses per day; BMI, body mass index.

Table 1. Clinical and metabolic characteristics of adolescent boys.

	Mean/(median)	**SD/(Q1-Q3)**
n	164	
Age (years)	16.7	1.8
BMI (Kg/m^2)	26	5
Waist (cm)	84	13
DBP (mmHg)	72	9
SBP (mmHg)	116	12
Glucose (mmol/l)	4.5	0.6
Insulin (mU/l)	(6.3)	(4.3-8.3)
HOMA-IR	(1.3)	(0.8-1.8)
TG (mmol/l)	(0.9)	(0.6-1.2)
TC (mmol/l)	4.1	0.9
HDL-C (mmol/l)	1.1	0.3
LDL-C (mmol/l)	2.6	0.9
CETP (%/ml.h)	151	36

SD, standard deviation; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; HOMA, homeostasis model assessment; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein, CETP, cholesteryl ester transfer protein. Data are expressed as mean and standard deviation or median and Q1-Q3, depending on parametric or non parametric distribution, respectively.

Table 2

Table 2. Clinical and metabolic characteristics of adolescent boys according to the presence of overweight and obesity.

	Normal Weight	Overweight	Obese	Significance
n (%)	84 (51.2)	37 (22.6)	43 (26.2)	
Age (years)	16.6±1.8	16.6±1.9	16.8±1.9	NS
BMI (Kg/m^2)	21.8±2.3	26.2±1.5	32.4±3.1	0.0001[a]
Waist (cm)	75±7	86±7	101±9	0.0001[a]
DBP (mmHg)	71±9	68±7	77±9	0.0001[b]
SBP (mmHg)	114±10	113±10	125±12	0.0001[b]
Glucose (mmol/l)	4.5±0.6	4.4±0.6	4.7±0.6	NS
Insulin (mU/l)	5.2 (3.6-7.1)	6.1 (4.4-7.7)	9.4 (6.4-12.7)	0.0001[b]
HOMA-IR	1.0 (0.7-1.5)	1.2 (0.9-1.6)	2.1 (1.2-2.7)	0.0001[b]
TG (mmol/l)	0.8 (0.6-1.1)	0.8 (0.6-1.1)	1.2 (0.9-1.6)	0,0001[b]
TC (mmol/l)	3.9±0.9	3.8±0.6	4.4±0.9	0.005[b]
HDL-C (mmol/l)	1.2±0.4	1.1±0.3	1.0±0.2	0.001[c]
LDL-C (mmol/l)	2.5±0.9	2.4±0.6	3.1±0.8	0.0001[b]

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; HOMA, homeostasis model assessment; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; NS, non significant. Data are expressed as mean±standard deviation or median (Q1-Q3), depending on parametric or non parametric distribution, respectively.

[a] Significance found between each group.

[b] Significance found comparing obese to normal weight and overweight subjects.

[c] Significance found comparing normal weight to obese subjects.

Table 3. Clinical and metabolic characteristics of adolescent boys according to CETP activity.

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	< median	**≥ median**	**Significance**
n	82	82	
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SBP (mmHg)	115±11	118±12	NS
Glucose (mmol/l)	4.5±0.6	4.6±0.7	NS
Insulin (mU/l)	6.0 (4.2-7.6)	6.5 (4.3-8.9)	NS
HOMA-IR	1.2 (0.8-1.6)	1.3 (0.9-1.9)	NS
TG (mmol/l)	0.8 (0.6-1.0)	1.0 (0.7-1.3)	0.001
TC (mmol/l)	3.8±0.8	4.3±0.9	0.005
HDL-C (mmol/l)	1.2±0.4	1.0±0.2	0.001
LDL-C (mmol/l)	2.4±0.8	2.9±0.9	0.001
TV>2 h [n,(%)]	63 (75.4)	70 (85.7)	0.08

Milk>3 glasses [n,(%)]	3 (3.7)	15 (18.3)	0.002

CETP, cholesteryl ester transfer protein; BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; HOMA, homeostasis model assessment; TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TV>2h, TV watching more than two hours daily; Milk>3 glasses, milk intake above three glasses per day; NS, non significant. Data are expressed as mean±standard deviation or median (Q1-Q3), depending on parametric or non parametric distribution, respectively.

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BMI	1.08	0.49	2.17	0.03	

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Figure
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