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Apolipoproteins and their association with cardiometabolic risk biomarkers in adolescents

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Abstract

Introduction: **the apoB/apo A-I ratio has been reported as an important predictor of cardiovascular risk, being superior to lipids, lipoproteins and conventional lipid ratios.**

Objective: **to investigate the association between apolipoproteins A-I and B, and the apolipoprotein B/apolipoprotein A-I ratio and cardiometabolic risk variables in adolescents.**

Methods: **this was a cross-sectional study including 104 adolescents of public schools in Recife during the months of March/April, 2013. Sociodemographic, anthropometric, clinical and biochemical variables were analysed. The apolipoproteins were analysed via Immunoturbidimetry.**

Results: **body mass index, waist circumference, waist circumference/height, triglycerides, cholesterol/HDL, and apolipoprotein B/apolipoprotein A-I declined with the progress of the percentile distribution of apolipoprotein A-I concentrations, while the HDL and apolipoprotein B increased between the first and last quartiles of the apolipoprotein A-I concentrations. Systolic blood pressure, body mass index, waist circumference, waist circumference/height, cholesterol, LDL, triglycerides, cholesterol/HDL, and LDL/HDL increased progressively in the quartile distribution of the concentrations of apolipoprotein B and apolipoprotein B/apolipoprotein A-I. Alfa-1-acid glycoprotein serum levels increased hand-in-hand with the percentile progression of apolipoprotein B.**

Conclusions: **the findings underline an important association of apolipoproteins A-I and B, and the apolipoprotein B/apolipoprotein A-I ratio and their clinical, biochemical and anthropometric cardiometabolic risk. However, prospective studies are important to evaluate the pertinence of implementing these markers in the clinical practice.**

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Key words: *Apolipoprotein A-I. Apolipoprotein B. Dyslipidemias. Adolescent.*

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APOLIPOPROTEÍNAS Y SU ASOCIACIÓN CON BIOMARCADORES DE RIESGO CARDIOMETABÓLICO EN ADOLESCENTES

Resumen

Introducción: **la razón apo B/apo A-I sigue siendo reportada como un predictor importante de riesgo cardiovascular, superior a lípidos, lipoproteínas y razones lipídicas convencionales. Objetivo: investigar la asociación entre las apolipoproteínas A-I y B y la razón apolipoproteína B/apolipoproteína A-I con variables de riesgo cardiometabólico en adolescentes.**

Métodos: **estudio de corte transversal que incluye a 104 adolescentes de escuelas públicas de Recife, entre marzo/abril de 2013. Se evaluaron variables clínicas, bioquímicas, antropométricas y sociodemográficas. Se analizaron las apolipoproteínas por imunoturbidimetría.**

Resultados: **índice de masa corporal, circunferencia de la cintura, circunferencia de la cintura/altura, triglicéridos, colesterol/HDL y apolipoproteína B/apolipoproteína A-I mostraron una reducción con la progresión de la distribución en percentil de las concentraciones de apolipoproteína A-I, mientras que HDL y apolipoproteína B aumentaron entre el primero y el último cuartil de las concentraciones de apolipoproteína A-I. Tensión arterial sistólica, índice de masa corporal, circunferencia de la cintura, circunferencia de la cintura/altura, colesterol, LDL, triglicéridos, colesterol/HDL y LDL/HDL presentaron un aumento progresivo en la distribución en cuartiles de las concentraciones de apolipoproteína B y de la apolipoproteína B/apolipoproteína A-I. Los niveles séricos de alfa-1-glicoproteína ácida aumentaron paralelamente a la progresión en percentil de apolipoproteína B.**

Conclusiones: **los hallazgos evidencian una asociación importante de las apolipoproteínas A-I y B y de la razón apolipoproteína B/apolipoproteína A-I con biomarcadores clínicos, bioquímicos y antropométricos de riesgo cardiometabólico. Sin embargo, son recomendables estudios prospectivos para evaluar la pertenencia de la implementación de esos marcadores en la práctica clínica.**

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Palabras clave: *Apolipoproteína A-I. Apolipoproteína B. Dislipidemias. Adolescente.*

Abbreviations

AGP: Alfa-1-acid glycoprotein.
APO A-I: Apolipoprotein A-I.
APO B: Apolipoprotein B.
BMI: Body mass index.
DBP: Diastolic blood pressure.
HDL: High-density lipoprotein.
LDL: Low-density lipoprotein.
SBP: Systolic blood pressure.
TAG: Triglycerides.
TC: Total cholesterol.
WC: Waist circumference.

Introduction

Although the atherosclerotic disease in general prevails in the adult age, cardiovascular risk markers have been identified in children[1] and adolescents[2], indicating their need to be investigated in these age ranges[3]. Regarding the lipid profile, it has been observed that the risk of atherosclerotic disease seems to be more closely related to the number of circulating atherogenic particles that contact and go through the arterial wall, than with the isolated concentrations of cholesterol contained in those lipoprotein fractions[4]. Consequently, measuring blood concentrations of apolipoproteins B and A-I has been considered for expressing more appropriately the balance of atherogenic and antiatherogenic particles[5].

Apolipoproteins are structural and functional proteins of the lipoprotein particles[6], which perform important functions for the lipoprotein metabolism as carriers of these hydrophobic molecules in the plasma aqueous medium, binding to the specific receptors on the cell surface to conduct the lipids correctly to the target organs and tissues of the organism. They also activate or inhibit the enzymes involved in the lipid metabolism[4,7].

Apolipoprotein A-I (apo A-I) is largest structural protein component of the high-density lipoprotein (HDL). It is responsible for stimulating the reverse cholesterol transport, removing the exceeding cholesterol from the tissues and redirecting it to the liver[8], and it may also be responsible for the anti-inflammatory and anti-oxidant properties of the HDL[4].

Apolipoprotein B (apo B) accounts for approximately 90% of the protein in the low-density lipoprotein (LDL), and it is the principal functional protein for carrying cholesterol to the peripheral cells. It is present in kilomicrons (apo B-48), in the very low density and intermediary lipoproteins, and in the LDL such as apo B-100[7], correlating with the non-HDL cholesterol level[1].

The apoB/apoA-I ratio, therefore, reflects the cholesterol balance of the potentially atherogenic and anti-atherogenic lipoprotein particles. Consequently, its high value would indicate an increased tendency to cholesterol deposition, endothelial dysfunction and, as a result, higher risk of atherogenesis[6,8]. This is why the apoB/apo A-I ratio has been reported as an important predictor of cardiovascular risk, being superior to lipids, lipoproteins and conventional lipid ratios, such as total cholesterol/HDL[9].

Considering the magnitude of the coronary arterial disease at earlier ages, all over the world, this study aimed at investigating the association of both apolipoproteins A-I and B and the apoB/apoA-I ratio with cardiometabolic risk variables in adolescents of public schools in Recife, Northeast Brazil.

Materials and methods

Study Design and Population

The investigation derived from a larger project titled "Dyslipidemia and its association with excessive weight, sedentary lifestyle and oxidative stress in a cohort of school students in Recife, PE". An evaluation was made of the anthropometric, lipid profile, food consumption and lifestyle parameters, in addition to socioeconomic and demographic aspects, in the baseline of this project.

The outline of the research comprised a cross-sectional study, nested in this cohort, the eligible population of which consisted of adolescents of both genders from 12 to 19 years of age, recruited in public schools of Recife, in the period from March to April 2013.

In order to size the sample, a pilot study was undertaken, and a prevalence was found showing a 50.0% increase in triglycerides (TAG) serum concentrations between the first and the last quartile of the apoB/apoA-I index distribution. In defining the sample size, an equation was used to calculate the sample with infinite population[10] [$n = z^2 \times pq/d^2$], where z = confidence interval (1.96), p = estimated prevalence (50.0%), and d = margin of error (14.0%). As this is a "finite" population, the sample "n" was adjusted according to the equation [$n = n/1 + (n/N)$][11], where n = sample "n" (49), and N = population size (409). Then, a correction was made due to the effect of the sample design (factor 2.1) per cluster, resulting in a minimum number of sample units of 92 individuals. In order to repair any losses, a choice was made to correct the sample size by 10.0% [100/(100-10)], resulting in a final sample of 103 adolescents.

Evaluation Techniques and Methods

The researchers conducted the fieldwork supervision, and a previously trained technical team collected the data. The technical team is experienced in checking clinical and anthropometrical measures, handling biological materials and delivering the specific questionnaires.

Clinical and Biochemical Variables

Systolic blood pressures (SBP) and diastolic blood pressures (DBP) were checked by the auscultation method. Two right-arm measurements were made per adolescent in 5-minute intervals. SBP and DBP were registered in the first and fourth Korotkoff's phases, respectively. The values acquired in the second check were considered and classified according to the 1st Brazilian Guideline for the Prevention of Atherosclerosis in Children and Adolescents[12].

As to the biochemical analyses, approximately 5 mL of blood were drawn from each student by venous puncture after a 10- to 12-hour fasting. The blood was stored in dry vials in a room prepared in advance, in each school. The materials used for collecting and processing were all disposable and sterile. The vials were stored in Styrofoam boxes containing reusable ice packs, sealed and transported for processing and analysis in a clinical analysis laboratory. An analysis was made of the serum concentrations of glucose, TAG, total cholesterol (TC), LDL and HDL, apolipoprotein A-I, apolipoprotein B and alfa-1-acid glycoprotein (AGP). Glucose, TAG, TC and HDL serum levels were determined by means of an enzymatic method. The LDL fraction was calculated with Friedewald's formula [LDL=TC - HDL - TAG/5]. This formula is valid for plasma concentrations of TAG <4.5 mMol/L. Above this, the LDL values are underestimated[12].

The reference values used to diagnose dyslipidemias were preconized by the 1st Brazilian Guideline for the Prevention of Atherosclerosis in Children and Adolescents[12]. Serum levels of TC >4.4 mMol/L, LDL >3.4 mMol/L and TAG >1.5 mMol/L were all classified as increased values. Serum concentrations of HDL <1.2 mMol/L are deemed to be below the desirable values.

The diagnostic criteria of *diabetes mellitus* were those preconized by the American Diabetes Association[13], which views *diabetes* whenever fasting glycaemia is ≥7.0 mMol/L, after a fasting period of at least 8 hours. Fasting glycaemia values between >5.6 e <7.0 mMol/L are deemed an increased risk for *diabetes*. Apolipoproteins B and A-I, as well as alfa-1-acid glycoprotein were analysed using kits based on the Immunoturbidimetric method (Randox, UK).

Anthropometric Variables

The anthropometric evaluation consisted of measuring two values of weight, height and waist circumference, and the averages of these two values were used. For data consistency purposes, differences greater than 100 g for weight, 0.5 cm for height and 0.3 cm for waist circumference in the measured values were not taken into account.

The measurements of weight and height were done according to the original technique recommended by Lohman, Roche and Martorell[14]. The body mass was obtained from a digital electronic scale, brand Plenna-MEA-03140®, with maximum load of 150 Kg and precision of 100 g. The height was measured with a Stanley® metric measuring tape with 1.0 mm precision and 0.5 cm accuracy. The waist circumference (WC) was obtained from the middle point between the last costal arch and the iliac crest using a flexible and short measuring tape, without compressing the tissues[15].

The overweight diagnosis was achieved by using the body mass index (BMI), and the result found was interpreted in accordance with the values indicated by WHO-2007[16], considering gender and age. The abdominal obesity diagnosis was achieved by evaluating the WC and the WC (cm)/Height (cm) ratio. For the classification of waist circumference, the cutoff point used for definition of abdominal obesity was value ≥percentile 80 adjusted for age and gender[15]. Regarding WC/Height, the cutoff point used for the definition of abdominal obesity was value ≥0.5[17].

A Maltron BF-906® (Maltron Int`l Ltd, Essex, UK) was used to measure bioimpedance with four electrodes at a frequency of 50 Hz, alternating current. The evaluation consists of performing the technique twice[18]. The equipment provides the percentage of fat directly by means of equations previously programmed by the manufacturers of the instrument.

Sociodemographic and Lifestyle Variables

Age, gender, education level and socioeconomic classification data were collected. The education level of the adolescents was determined by the number of full years of study, based on the grade in which the adolescent was enrolled. The parents' education level was determined by the number of full years of study, and classified according to the criteria of the Brazilian Association of Research Companies[19], grouped as completed elementary education, secondary education, and higher education.

In determining the family's socioeconomic level, the "Brazilian Economic Ranking Criteria" were used, as established by Brazilian Association of Research Companies[19]. This system uses a scoring scale, obtained by adding the scores related to the household appliances owned by the head of the family, and by his or her level of education, consequently ranking the population in classes A1, A2, B1, B2, C1, C2, D and E, in descending order, starting at the highest purchasing power.

The adolescents who claimed to smoke a number of cigarettes >5 per day[20] was classified as smoker. The levels of physical activity were classified as follows: a) underactive or sedentary – individuals who accumulated <300 minutes of weekly physical activity; b) sufficiently active – individuals who accumulated >300 minutes/week[21].

Statistical analysis

The Epi Info program, version 6.04b (WHO/CDC, Atlanta, GE, USA) was used, and the data were typed in double data entry, and data consistency was verified by module *validate*. The statistical analyses were done using the *Statistical Package for Social Sciences* – SPSS for Windows, version 13.1 (SPSS Inc., Chicago, IL, USA).

The distribution normality of the continuous variables was tested using Kolmogorov Smirnov's test. The concentrations of apoB, apoA-I, apoB/apoA-I rate, AGP, HDL and glucose, as well as the percentage of fat were normally distributed and were described in the form of averages and standard deviations. The variables that were not normally distributed underwent a logarithmic transformation (Ln), and their normality was retested. Only LDL fraction concentrations were normally distributed and were described in the form of geometrical averages and confidence intervals of 95%. The other variables still presenting a non-Gaussian distribution were described in the form of their medians and interquartile ranges.

The adolescents were classified into four groups according to the percentile distribution of apolipoproteins A-I and B serum concentrations, and the apoB/apoA-I ratio. The analysis of variance (One-Way ANOVA) was used to compare the averages of the calculated quartiles when the homoscedasticity criteria and normal distribution were met, and Bonferroni's test was used later.

Kruskal Wallis' and Mann Whitney's "U" tests were used when normality or homoscedasticity criteria were not met. In describing the proportions, the binomial distribution approximated the normal distribution by the confidence interval of 95%. In the statistical inference tests, proportions were compared by Pearson's Chi-square test. The significance level adopted for rejecting the null hypothesis was 5.0%.

Ethical Aspects

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Committee of Ethics in Research with Human Beings of Lauro Wanderley University Hospital – CEP/HULW at the Federal University of Paraíba [*Comitê de Ética em Pesquisa em Seres Humanos do Hospital Universitário Lauro Wanderley – CEP/HULW da Universidade Federal da Paraíba*], and was approved as per Record CEP/HULW No. 723/10.

The adolescents and their guardians were informed in advance about the objectives and methods to be adopted in the research, when contacted before the day of the collection by the technical team. Written informed consent was obtained from all subjects.

Results

The sample consisted of 104 individuals. Both genders were proportionally distributed (p=0.845). The median age was 15 years (Interquartile Range=2.0). The minimum age was 12 years, and the maximum age was 19 years. Regarding socioeconomic and lifestyle characteristics, 50.7% of the fathers ($CI_{95\%}$: 38.8-62.5), and 54.7% ($CI_{95\%}$: 43.6-65.3) of the mothers of the studied adolescents did not complete their secondary education, and 77.0% ($CI_{95\%}$: 66.7-84.8) of the families were part of social classes C or D. Only three adolescents (2.9%, $CI_{95\%}$: 0.8-8.8) were classified as smokers, and 64.4% were considered underactive or sedentary ($CI_{95\%}$: 54.4-73.4).

The percentile distribution of apolipoproteins A-I and B, as well as the apoB/ApoA-I ratio did not vary as a function of variables gender, age, parental level of education, social class and physical activity. An observation regarding the mothers' education level, however, was that a higher education level was associated with lower serum concentrations of ApoA-I and higher concentrations of the apoB/apoA-I ratio. Overweight was found in 35.6% ($CI_{95\%}$: 26.6-45.6) of the sample, of which 14.4% ($CI_{95\%}$: 8.6-23.0) were classified as obese, according to the BMI for age and gender. Abdominal obesity was found in 27.9% ($CI_{95\%}$: 19.7-37.7) of the individuals, according to their waist circumference, and in 25.0% ($CI_{95\%}$: 17.3-34.6) of the adolescents, according to their waist circumference/height ratio. High systolic and/or diastolic blood pressure levels were found in 15.4% ($CI_{95\%}$: 9.3-24.1) of the sample. There were no cases of fasting glycaemia suggesting *diabetes mellitus* (>7 mMol/L), but 4.8% ($CI_{95\%}$: 1.8-11.4) of the individuals were found glucose intolerant (>5,6 e <7 mMol/L) (Table I).

The demographic, anthropometric, clinical and biochemical characteristics of the participants, according to the percentile distribution of apoA-I, apoB and apoB/apoA-I ratio concentrations, are given in tables II, III and IV, respectively.

BMI, WC, WC/Height and TAG values and TC/HDL and apoB/apoA-I ratios expressed a statistically significant reduction with the progress of the percentile distribution of apoA-I concentrations. However, the averages of HDL and apoB, on their turn, increased significantly from the first to the last quartile of the apoA-I concentrations. Both the BMI, WC and WC/Height anthropometric variables, and the SBP, TC, LDL, TAG, as well as the CT/HDL and LDL/HDL indices increased significantly over the distribution in the quartiles of apoB and apoB/apoA-I ratio.

AGP serum levels varied only within the quartiles of apoB, increasing *pari passu* with the progress of the percentile distribution. Gender, age, fat percentage and glycaemia in the adolescents did not show any significant differences within the evaluated quartiles of the isolated apolipoproteins and apoB/apoA-I ratio.

Table I
Sociodemographic and lifestyle characteristics of the adolescents according to apo A-I, apo B and apoB/apoA-I ratio serum concentrations. Recife, Brazil

Variables	*Categories*	*n*	*%*	*ApoA-I*			*ApoB*			*ApoB/ApoA-I*		
				Mean	*SD**	*p§*	*Mean*	*SD**	*p§*	*Mean*	*SD**	*p§*
Gender	Male	51	49·0	243·5	64·4	0·902	208·8	47·9	0·270	0·89	0·21	0·422
	Female	53	51·0	241·8	78·2		197·4	55·9		0·86	0·24	
	Total	104	100·0									
Age (years)	12 to 15	72	69·2	247·9	68·0	0·261	206·8	51·3	0·268	0·87	0·21	0·675
	16 to 19	32	30·8	230·8	78·4		194·5	54·0		0·89	0·26	
	Total	104	100·0									
Father's Schooling†	<Elementary School	37	50·7	247·1	74·8	0·888	204·6	43·5	0·664	0·87	0·20	0·667
	High School / Higher Education	36	49·3	244·7	67·6		209·8	58·4		0·90	0·28	
	Total	73	100·0									
Mother's Schooling†	<Elementary School	47	54·7	268·3	60·3	0·006	207·4	42·7	0·631	0·80	0·18	0·001
	High School / Higher Education	39	45·3	230·3	64·3		212·2	55·6		0·96	0·26	
	Total	86	100·0									
Social Class†	B1 or B2	21	23·1	229·5	67·8	0·232	189·2	51·1	0·104	0·86	0·18	0·735
	C1, C2 or D	70	76·9	250·2	69·8		209·2	48·3		0·88	0·24	
	Total	91	100·0									
Physical Activity‡	Underactive	67	64·4	246·1	72·7	0·514	205·3	56·1	0·550	0·87	0·21	0·604
	Active	37	35·6	236·5	69·6		198·9	44·7		0·89	0·26	
	Total	104	100·0									

*SD, standard deviation; †Brazilian Association of Research Companies[19]; ‡Pate, Freedson, Salli, Taylor, Sirard, Trost *et al*.[21]; §*Student*'s "t"-test for unpaired data.

Discussion

In the studied population of adolescents, apolipoproteins B and A-I, as well as the apoB/apoA-I ratio, were significantly and simultaneously associated with the traditional variables of cardiometabolic risk, such as BMI, WC and WC/Height, in addition to HDL and TAG serum levels.

Of the analysed distributions, apolipoprotein B was associated with the largest number of variables that, in addition to anthropometric, BMI, WC and WC/Height variables, also included all evaluated lipidemia and lipid ratios, as well as systolic blood pressure levels, and the AGP inflammatory marker. Likewise, the concentrations of the apoB/apoA-I ratio also were associated with the lipid profile, except the serum levels of apoB itself, in addition to being associated with the systolic and diastolic blood pressure levels.

The results found in the studied population of adolescents matched previous clinical and epidemiological studies determining that the metabolism of apolipoprotein is closely related to cardiovascular disease risk factors[22-24]. In the study Effect of Potentially Modifiable Risk Factors Associated with Myocardial Infarction in 52 Countries – INTERHEART[23,25], which studied approximately 30 thousand individuals of 52 countries, a high apoB/apoA-I ratio was the strongest risk factor for acute myocardial infarction. This predictor contributed to approximately 50% of the risk for cardiovascular events, in addition to being the most prevalent risk factor of all the remaining investigated conventional factors (smoking, hypertension, diabetes, stress and abdominal obesity), irrespective of age, gender, ethnicity and other lipids or lipid indices.

At different moments of the Apolipoprotein-related Mortality Risk Study – AMORIS[26,27], which investigated approximately 170 thousand Swedish individuals, it was found that apo B was a cardiovascular risk factor superior to LDL, irrespective of the gender. On its turn, the apoB/apoA-I ratio was the strongest risk fac-

Table II
Demographic, anthropometric, clinical and biochemical characteristics of the adolescents, according to their apo A-I serum concentrations. Recife, Brazil

Variables[1]	Apo A-I quartiles								p
	I (n = 26)		II (n = 27)		III (n = 26)		IV (n = 25)		
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Girls (%)	57·7	-	48·1	-	34·6	-	64·0	-	0·169‖
Age (years)	15·0*	2·0†	15·0*	2·0†	14·5*	1·5†	15·0*	2·0†	0·341¶
BMI (kg/m²)	24·1*a	7·3†	22·4*a	8·0†	23·5*a	10·3†	19·6*b	3·0†	0·007¶
WC (cm)	72·7*a	15·1†	74·0*a	23·7†	70·7*a	25·4†	64·1*b	7·1†	0·002¶
WC/Height	0·5*a	0·1†	0·4*a	0·1†	0·5* a	0·2†	0·4*b	0·1†	0·005¶
Fat (%)	28·7	12·9	30·7	14·2	28·6	13·6	26·2	9·3	0·719**
SBP (mmHg)	110·0*	10·0†	120·0*	10·0†	120·0*	20·0†	110·0*	10·0†	0·073¶
DBP (mmHg)	70·0*	20·0†	70·0*	10·0†	70·0*	10·0†	70·0*	15·0†	0·343¶
Glucose (mmol/L)	4·6	0·5	4·7	0·5	4·8	0·5	4·5	0·5	0·205**
TC (mmol/L)	4·3	0·7	4·4	1·1	4·4	0·7	4·1	0·8	0·588**
HDL (mmol/L)	1·1d	0·2	1·1d	0·2	1·1d	0·1	1·3e	0·12	0·000**
LDL (mmol/L)	2·4‡	2·2-2·6§	2·4‡	2·0-2·7§	2·5‡	2·3-2·8§	2·3‡	2·0-2·5§	0·744**
TAG (mmol/L)	1·5*a	1·1†	1·6*a	1·3†	1·6*a	1·2†	0·9*b	0·3†	0·006¶
TC/HDL	3·5*a	1·6†	3·7*a	1·7†	3·6*a	1·8†	3·1*b	0·7†	0·011¶
LDL/HDL	2·0*	0·9†	1·9*	1·1†	2·1*	1·1†	1·7*	0·7†	0·670¶
Apo B (g/L)	1·5d	0·5	2·1e	0·4	2·3e	0·3	2·2e	0·4	0·000**
ApoB/A-I	1·0a	0·2	0·9a	0·2	0·8b	0·1	0·7c	0·1	0·000**
AGP (g/L)	0·7	0·2	0·8	0·2	0·8	0·1	0·8	0·2	0·133**

[1]BMI, Body Mass Index; WC, Waist Circumference; WC/Height, Waist Circumference/Height; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TC, Total Cholesterol; TAG, Triglycerides; ApoB, Apolipoprotein B; ApoA-I, Apolipoprotein A-I; AGP, a_1-acid glycoprotein; *Median; †Interquartile Range; ‡Geometric Mean; §95% Confidence Interval; ‖*Pearson*'s Chi-square test; ¶*Kruskal-Wallis*' test; **One-Way ANOVA; a,b,c letters ≠ mean averages ≠ at significance level 5.0% (Mann-Whitney's "U" test); d,e, letters ≠ mean averages ≠ at significance level 5.0% (Bonferroni's test).

tor for myocardial infarction, when compared to the TC/HDL and LDL/HDL ratios, especially when the lipid levels were within the range of desirable values.

A prospective study representative of the North American population found that the apoB/apoA-I ratio was significantly associated with mortality due to coronary artery disease, irrespective of other traditional risk factors, and that it is a better predictor for cardiovascular disease mortality, when compared to the TC/HDL ratio[9].

Exposure to an unfavourable lipid profile from an early age might induce arterial effects in the long run[28]. According to The Cardiovascular Risk in Young Finns Study[29], the levels of apo B and apo A-I and the apoB/apoA-I ratio in adolescence were predictive for changes in the arterial health in adulthood, such as thicker intimal carotid layer and endothelial dysfunction. These relations did not depend on the concentrations of apolipoproteins in the adult life. It was also found that a high apoB/apoA-I ratio was superior to the conventional lipid measures (LDL, HDL, LDL/HDL, non-HDL/HDL) in predicting the abnormal thickness of the carotid intimal layer. This study, therefore, underlines adolescence as a critical period in life, as being exposed to risk factors may produce long-lasting adverse effects on the arterial health, which corroborates the relevance of studies for this age group, such as the results observed in our population of adolescents in Recife.

The Bogalusa Heart Study[4] also demonstrated that the thickness of the carotid intimal layer in adults increases significantly over the quartiles of apo B levels and apoB/apoA-I index in childhood and adolescence, which, along with the non-HDL, LDL levels, and the TC/HDL ratio, emerged as significant predictors of this harm to someone's adult life.

Despite methodological and age range differences, similar associations of the lipid variables and both

Table III
Demographic, anthropometric, clinical and biochemical characteristics of the adolescents, according to their apo B serum concentrations. Recife, Brazil

Variables[1]	Apo B Quartiles								p
	I (n = 26)		II (n = 26)		III (n = 26)		IV (n = 26)		
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Girls (%)	57·7	-	42·3	-	57·7	-	46·2	-	0·580‖
Age (years)	15·0*	2·0†	15·0*	2·0†	15·0*	2·0†	15·0*	1·2†	0·651¶
BMI (kg/m²)	21·8a	4·3	21·0a	5·3	22·3a	5·0	27·2b	6·7	0·000**
WC (cm)	68·1*c	11·7†	66·7*c	12·7†	66·5*c	16·0†	87·0*d	30·9†	0·002¶
WC/Height	0·4*c	0·1†	0·4*c	0·1†	0,4*c	0·1†	0·5*d	0·2†	0·000¶
Fat (%)	27·2	13·6	25·6	17·6	28·1	9·6	33·2	7·9	0·177**
SBP (mmHg)	110·0*c	10·0†	110·0*c	10·0†	120·0*c	10·0†	120·0*d	22·5†	0·001¶
DBP (mmHg)	70·0*	20·0†	70·0*	12·5†	70·0*	12·5†	80·0*	20·0†	0·052¶
Glucose (mmol/L)	4·6	0·5	4·7	0·5	4·5	0·5	4·7	0·5	0·377**
TC (mmol/L)	3·8*c	0·7†	4·0*c	0·6†	4·1*c	0·3†	4·9*d	1·3†	0·000¶
HDL (mmol/L)	1·2*c	0·3†	1·2*c	0·1†	1·2*c	0·3†	1·1*d	0·1†	0·001¶
LDL (mmol/L)	2·1‡a	1·8-2·4§	2·1‡a	1·9-2·3§	2·3‡a	2·1-2·5§	3·0‡b	2·7-3·3§	0·000**
TAG (mmol/L)	1·4a	0·7	1·1a	0·4	1·3a	0·7	2·0b	0·7	0·000**
TC/HDL	3·3*c	1·3†	3·1*c	0·4†	3·3*d	1·2†	4·8*e	1·4†	0·000¶
LDL/HDL	1·8*c	0·8†	1·8*c	0·4†	1·9*c	0·6†	2·8*d	1·2†	0·000¶
Apo A-I (g/L)	1·7a	0·7	2·5b	0·5	2·8b	0·5	2·7b	0·5	0·000**
ApoB/A-I	0·9a,b	0·2	0·8a	0·3	0·8a	0·2	1·0b	0·2	0·004**
AGP (g/L)	0·7a	0·2	0·7a,b	0·2	0·8a,b	0·2	0·8b	0·2	0·038**

[1]BMI, Body Mass Index; WC, Waist Circumference; WC/Height, Waist Circumference/Height; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TC, Total Cholesterol; TAG, Triglycerides; ApoB, Apolipoprotein B; ApoA-I, Apolipoprotein A-I; AGP, a_1-acid glycoprotein; *Median; †Interquartile Range; ‡Geometric Mean; §95% Confidence Interval; ‖*Pearson* 's Chi-square test; ¶*Kruskal-Wallis*' test; **One-Way ANOVA; a,b. letters ≠ mean averages ≠ at significance level 5.0% (Bonferroni's test); c,d,e, letters ≠ mean averages ≠ at significance level 5.0% (Mann-Whitney's "U" test).

systolic and diastolic blood pressure levels and the apoB/apoA-I ratio, found in the adolescents studied in Recife, were described in a study involving the adult Chinese population[6]. This study revealed that the average of the apoB/apoA-I ratio increased according to the number of metabolic syndrome components and that, even after the exclusion of the TAG and HDL variables, this correlation remained significant, indicating that the association of the apoB/apoA-I ratio and metabolic syndrome did not depend on the lipid components. In addition, there was a positive and significant correlation between the apoB/apoA-I ratio and variables WC, TAG, SBP and DBP, and a negative and significant correlation between the apoB/apoA-I ratio and HDL.

Overweight is increasing all over the world and has become a serious public health problem. The prevalence of obesity in childhood more than doubled in the past 15 years in many regions of the world[30], and has been described in the literature as the influence of the early nutritional status as the determining factor for the nutritional status in adulthood[31].

It is known that overweight in younger ages may lead to the early development of cardiovascular disease risk factors. The results of our investigation indicate that, in adolescence, the BMI, WC and the WC/Height ratio tend to increase according to the distribution of the apoB/apoA-I index, underlining again that overweight is consistently associated with cardiometabolic risk.

A large number of risk factors for cardiovascular disease, such as dyslipidemia, diabetes and arterial hypertension are acquired during childhood and adolescence, and tend to persist throughout life. The initial alterations of these factors, even if they are minor, associated with the distribution of body fat, determine an unfavourable cardiovascular profile for these young individuals[31].

Table IV
Demographic, anthropometric, clinical and biochemical characteristics of the adolescents, according to their apoB/apoA-I serum concentrations. Recife, Brazil

Variables[1]	Apo B/A-I Quartiles								p
	I (n = 26)		II (n = 26)		III (n = 26)		IV (n = 26)		
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
Girls (%)	53·8	-	65·4	-	53·8	-	38·5	-	0·249‖
Age (years)	15·0*	2·0†	15·0*	2·0†	15·0*	2·0†	15·0*	2·0†	0·823¶
BMI (kg/m²)	19·3*a	3·4†	20·5* a	5·2†	24·1*b	8·1†	25·4*b	9·3†	0·000¶
WC (cm)	64·0*a	7·3†	66·0*a	8·3†	74·7*b	23·2†	84·0*b	27·0†	0·000¶
WC/Height	0·4*a	0·1†	0·5*a	0·1†	0·5*b	0·1†	0·5*b	0·2†	0·000¶
Fat (%)	24·9	10·6	25·8	13·6	33·9	13·4	29·4	11·3	0·064**
SBP (mmHg)	110·0*a	10·0†	110·0*a	10·0†	120·0*b	22·5†	120·0*b	10·0†	0·001¶
DBP (mmHg)	70·0*a	10·0†	70·0*a,c	12·5†	80·0*b	12·5†	70·0*c	10·0†	0·008¶
Glucose (mmol/L)	4·6	0·2	4·7	0·5	4·5	0·4	4·7	0·5	0·314**
TC (mmol/L)	3·9*a	0·4†	3·9*a	0·6†	4·4*b	1·0†	4·5*b	1·7†	0·000¶
HDL (mmol/L)	1·3d	0·1	1·2d	0·1	1·1e	0·1	1·1e	0·2	0·000**
LDL (mmol/L)	2·1‡d	2·0-2·3§	2·1‡d	1·8-2·3§	2·6‡e	2·4-2·9§	2·7‡e	2·4-3·1§	0·000**
TAG (mmol/L)	0·9*a	0·12†	0·9*a	0·7†	1·6*b	0·6†	2·0*b	0·8†	0·000¶
TC/HDL	3·0*a	0·6†	3·1*a	0·5†	4·4*b	1·5†	4·8*b	2·1†	0·000¶
LDL/HDL	1·7*a	0·6†	1·8*a	0·3†	2·5*b	1·1†	2·7*b	1·6†	0·000¶
Apo A-I (g/L)	3·2*a	0·3†	2·5*b	0·6†	2·5*b	0·9†	1·9*c	1·3†	0·000¶
ApoB (g/L)	2·1*	0·3†	1·9*	0·4†	2·3*	0·9†	2·1*	1·4†	0·051¶
AGP (g/L)	0·8	0·2	0·8	0·2	0·8	0·2	0·8	0·2	0·742**

[1]BMI, Body Mass Index; WC, Waist Circumference; WC/Height, Waist Circumference/Height; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; TC, Total Cholesterol; TG, Triglycerides; ApoB, Apolipoprotein B; ApoA-I, Apolipoprotein A-I; AGP, a_1-acid glycoprotein; *Median; †Interquartile Range; ‡Geometric Mean; §95% Confidence Interval; ‖*Pearson*'s Chi-square test; ¶*Kruskal-Wallis*' test; **One-Way ANOVA; a,b,c, letters ≠ mean averages ≠ at significance level 5.0% (Mann-Whitney's "U" test); d,e, letters ≠ mean averages ≠ at significance level 5.0% (Bonferroni's test).

Additionally, it is worth to note that 64.4% of the studied adolescents of our sample in Recife were underactive or sedentary, thus characterizing an important percentage of young individuals taking an additional risk behaviour in terms of cardiovascular prognosis.

The role of inflammation in the pathogenesis of atherosclerosis and its complications also deserves to be highlighted[30]. A chronic inflammatory state, developed because of the summation of metabolic disorders and environmental stimuli, induces an acute phase response favouring abnormalities that include alterations in the metabolism of lipids and lipoproteins[32]. Onat and Hergenç[33] found both in the adult and in the elderly population in Turkey that the prevalence of a pro-inflammatory and pre-oxidative state relates to a high prevalence of obesity and metabolic syndrome. In these circumstances, HDL and apoA-I particles lose their anti-inflammatory and atheroprotective properties, and the balance between lipoproteins containing apoB and the reverse cholesterol transport slants towards the development of cardiovascular disease and/or diabetes.

In the studied population, there was a tendency to a significant increase in alpha-1-acid glycoprotein, along with the evolution of the apoB quartiles, emphasizing the association between the largest number of pro-atherogenic particles in the plasma and a growing inflammatory state favouring arterial lesions. This is why the evaluation of inflammatory markers, such as the AGP, might be considered a relevant tool to add useful information to traditional cardiovascular risk markers, such as the lipid profile.

The study of apolipoproteins has methodological advantages to identify other variables of the lipid profile and to estimate the LDL fraction using Friedewald's equation[4]. It is possible to accurately measure apolipoproteins directly in the plasma using internationally standardized methods[34,35], which do not require pre-

vious fasting[4,9], and the relevant results show little biological variation and reduced fluctuation in response to the effect of metabolic control. However, the absence of common sense regarding the reference values and the therapeutic objectives, on their turn, may limit the regular use of the apoB/apo A-I ratio.

As a limitation to this study, it is possible to mention that the cross-sectional design does not allow inferring possible causal relations between apolipoproteins and the cardiometabolic profile – only allowing associations. Prospective studies in this line, then, are extremely recommended. Moreover, it was not possible to assess the role of regular diet in the studied population, as this variable would exert, in theory, important impact on the individuals' lipid profile.

In short, the apolipoproteins A-I and B, and the apoB/apoA-I ratio serum concentrations were importantly associated with conventional anthropometric and biochemical variables of cardiovascular risk in the analysed adolescent population. It is suggested that additional investigations be conducted, especially with a prospective outline, in order to enrich the existing knowledge about the behaviour and contribution of these variables to other young populations that might be exposed to future adverse events, subject to the appropriate control.

In the assessment of the cardiometabolic risk, it is necessary to understand the summation of factors predisposing the population to this harm, depicting a global profile of higher predictive value. In this sense, such findings emphasize that measuring serum apolipoproteins during adolescence may constitute a useful clinical strategy for the identification and prompt intervention on individuals vulnerable to lifelong unfavourable cardiometabolic effects, providing benefits by offering measures to prevent atherosclerosis and cardiovascular disease.

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