Life Sciences 93 (2013) 64–68

Contents lists available at SciVerse ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Physical activity and cardiometabolic risk in male children and adolescents: The Balcarce study

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ARTICLE INFO

Article history:
Received 3 September 2012
Accepted 22 May 2013

Keywords:
Atherosclerosis
Lp-PLA$_2$
Physical activity

ABSTRACT

Aims: This study aims to evaluate the relationship between the amount of physical activity and different traditional and novel cardiometabolic risk factors, as well as atheroprotective agents, in male children and adolescents.
Main methods: Cross-sectional study. A total of 337 male children and adolescents aged 7–14 years old from the rural city of Balcarce, Buenos Aires, Argentina were studied.
Key findings: The main finding of the present study was that, in male children and adolescents, physical activity was inversely associated with lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) activity ($r = -0.39$, $p < 0.001$) and with cholesteryl ester transfer protein activity ($r = -0.23$, $p < 0.05$) apart from other proatherogenic agents after adjusting for age and BMI. Strikingly, among the parameters evaluated, overweight, hyperglycemia and Lp-PLA$_2$ activity resulted to be independently related to physical activity as shown by stepwise regression analysis.
Significance: The strong negative association between exercise and Lp-PLA$_2$ activity and the fact that the latter resulted to be the unique continuous variable that persisted associated with physical activity would add an additional benefit of exercise in early prevention of vascular inflammation and atherogenesis.

Introduction

Children and adolescents generally spend more time indoors using electronic media and less time playing outdoors or engaged in physical activities. Consequently, the reduction in energy expenditure attributed to decreased physical activity is likely to be one of the major factors contributing to the global epidemic of overweight and obesity (WHO, 2003). Moreover, this sedentary behavior may contribute to the development of a branch of clinical and subclinical metabolic abnormalities like hypertension, insulin resistance and dyslipidemia, all of them closely related to high risk of cardiovascular disease (Holst-Schumacher et al., 2009).

Cardiovascular disease is a multifactorial process known to begin very early in life, which emerges as a disbalance between pro- and antiatherogenic factors (Berenson et al., 1998). Among them, evaluation of overweight, particularly abdominal obesity, hypertension, hyperglycemia, dyslipidemia and the presence of the metabolic syndrome constitute a primary approach to assess risk (Reinehr et al., 2007). This evaluation could be widen by determining the so called novel risk factors such as cholesteryl ester transfer protein (CETP) (Quintão and Cazita, 2010), paraoxonase (PON) 1 (EC 3.1.8.1) (Mackness and Mackness, 2010) and lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) (EC 3.1.1.47) (Ikonomidis et al., 2011), which have been scarcely assessed in children and adolescents.

In the last years, many studies have been published supporting Lp-PLA$_2$ as a cardiovascular risk marker independent of and additive to traditional risk factors. High Lp-PLA$_2$ levels are present in inflamed, rupture-prone plaques, and it appears that Lp-PLA$_2$ is released from these plaques into the circulation (Corson et al., 2008). Moreover, Lp-PLA$_2$ has been recommended as an adjunct to traditional risk assessment in patients at moderate and high 10-year risk (Davidson et al., 2008).

Cardiorespiratory fitness has been associated with the prevention of atherosclerotic cardiovascular disease and with other health benefits (Lobelo et al., 2010). In this research, we aimed to study the relationship between the amount of physical activity and the novel risk factors apart from different traditional ones in male children and adolescents.

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http://dx.doi.org/10.1016/j.lfs.2013.05.021

Materials and methods

Study design and subjects

Data and samples were collected cross-sectionally during a period of 3 months from 349 male children and adolescents aged 7–14 years from different schools from the rural city of Balcarce, Buenos Aires, Argentina, at time of attending a scholar health control. That health control consists of a clinical assessment by a physician and laboratory analysis, and is mandatory for every child and adolescent prior to participation in soccer practice, which in Argentina is a popular sport played exclusively by males. From the total 349 individuals, 12 were discarded due to exclusion criteria, which consisted of: missing any required data, not being in fasting state, previous diagnosis of diabetes, hypothyroidism, renal, hepatic and cardiovascular disease, or any other chronic or acute disease, adherence to any special diet, smoking, the use of medication that could alter blood pressure, glucose or lipid metabolism and the informed consent not being signed. According to the National Institute of Statistics and Census of Argentina, the census carried out in 2001 revealed that the population of male subjects aged 6–14 years in Balcarce was 2766. Thus, in the present study, approximately 12% of the population was included (n = 337) (INDEC, 2009). Clinical examination and data collection from all the participants were carried out by a physician specialized in sport medicine. The study was approved by the Ethical Committee of "Dr. Felipe A. Fossati" Balcarce Hospital (Reference Number: 1821/10). Each parent and subject gave written informed consent after an explanation of the study and before its initiation.

Clinical characteristics

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. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Waist circumference measurement was taken at the level of the umbilicus and recorded to the nearest 0.1 cm. Blood pressure was measured using a random-zero sphygmomanometer and heart rate was taken at wrist level during 1 min after the participant had been seated at rest for 5 min. The physical examination also included determination of puberty stage according to Tanner's criteria. A personal interview was carried out by the same professional previously trained in order to estimate the amount of physical activity performed by each participant, which included evaluation of general training activities, sport practice and recreational actions. Upon enquiry none of the participants reported adherence to any special diet.

Samples collection

After a 12-hour overnight fast, venous blood was drawn from the antecubital vein and collected in clean tubes. Centrifugation was carried out at 1500 ×g and 4 °C for 15 min, and immediately used for glucose and lipid determinations. Aliquots were stored at −70 °C for other studies.

Physical activity questionnaire

A previously validated questionnaire was employed to estimate the amount of physical activity performed by children and adolescents (Barbosa et al., 2007). This questionnaire has demonstrated to be accurate and useful for epidemiological studies (Barbosa et al., 2007). The complete questionnaire included 13 items from which we specifically considered the following: item 4, related to transportation; item 7, related to other activities at school; item 8, related to out-school activities; item 10, related to vacation activities; and item 12, related to competitive sports. This questionnaire had been validated both in parts and as a whole (Barbosa et al., 2007). The aim of the shorter version was to evaluate every activity that made the participant increase his breathing rate or sweat instead of total movement. Those activities were principally related to transportation, to non-school activities (mainly sports) and to competitive sports. Activities related to daily living (sleeping, eating, watching television, etc.) or mandatory physical education that was the same for all individuals were excluded on purpose. Yearly physical activity was estimated including both scholar and leisure periods. The questionnaire was assessed to every children and adolescent and completed in a face to face interview by the same professional who had been previously trained. Physical activity amount was calculated as the summatory of all activities performed taken into account frequency and duration. Data of physical activity was expressed as hours per year.

Analytical determinations

Plasma glucose and lipoprotein profile were assayed by standardized techniques (Wiener Lab, Santa Fé, Argentina) in a Technicon RA-1000 system (Technicon Instruments Corporation, Tarrytown, New York, USA). Internal and external quality controls were assessed. Intra-assay CVs were: 0.9% for glucose, 0.7% for triglycerides, 0.9% for total cholesterol, 3.2% for high density lipoprotein-cholesterol (HDL-C) and 4.7% for low density lipoprotein-cholesterol (LDL-C). Inter-assay Cvs were: 1.9% for glucose, 1.7% for triglycerides, 1.0% for total cholesterol, 3.8% for HDL-C and 5.0% for LDL-C.

Cholesteryl ester transfer protein activity

CETP activity was determined in serum samples following the general procedure previously described (Lagrost et al., 1993; Lagrost, 1998) with few modifications. Briefly, the ability of serum to promote the transfer of tritiated cholesteryl esters from a tracer amount of biosynthetically labeled high density lipoprotein $(HDL)_3$ (^{3}H-CE-HDL_3) (NEN Life Science Products, Boston, USA) to serum apoprotein (apo) B-containing lipoproteins was evaluated. The original technique was modified by sterilizing ^{3}H-CE-HDL_3, a procedure that protected the lipoprotein from contamination. This determination was carried out in a subgroup of the studied population (n = 87) and all samples were evaluated within the same assay. Intra and inter-assay CVs were 4.9% and 6.0%, respectively.

Paraoxonase/arylesterase activity

The enzyme PON 1 was evaluated employing two different substrates: paraoxon (Sigma Chemical Co.; PON activity) and phenylacetate (Sigma Chemical Co.; ARE activity). Both activities were measured in serum samples following the method of Furlong et al. (1989). These determinations were carried out in a subgroup of the studied population (n = 87) and all samples were evaluated within the same assay. Intra- and inter-assay CVs for PON activity were 5.5% and 11.9%, respectively, and for ARE activity 4.8% and 12.6, respectively.

Lipoprotein-associated phospholipase A_2 activity

Lp-PLA_2 activity was measured following the radiometric assay described by Blank et al. (1983) with few modifications. The separation of the released radiolabeled acetate from the lipid substrate was carried out by phase–phase partitioning and radioactivity in the aqueous phase was then measured. This determination was carried out in a subgroup of the studied population (n = 87) and all samples were evaluated within the same assay. Intra- and inter-assay CVs were 6.4% and 14.5%, respectively.

Definition of metabolic situations

Overweight was defined based on BMI according to the international survey published by Cole et al. (2000). Abdominal obesity was defined as waist circumference above the 90th percentile as proposed by Cook et al.

(2003) adjusted by age and sex following the procedures proposed by Eisenmann (2005) and Davies and Eisenmann (2006) to obtain a Z score. Hypertension was defined according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (2004) which employs age, gender and height Z score obtained from CDC growth charts (CDC, 2009). Hyperglycemia was defined as glucose plasma levels above 5.55 mmol/l (Genuth et al., 2003; Reinehr et al., 2007). Hypertriglyceridemia was defined as triglyceride levels above or equal to 1.24 mmol/l (Kunze and Wabitsch, 2011). Hypercholesterolemia was defined as low density lipoprotein-cholesterol (LDL-C) levels above or equal to 2.60 mmol/l for children aged 4 to 7 and 2.86 mmol/l for children aged 8 to 15 years old (Kunze and Wabitsch, 2011). Hypoalphalipoproteinemia was defined as high density lipoprotein-cholesterol (HDL-C) levels below 1.04 mmol/l (Kunze and Wabitsch, 2011). Dyslipidemia was defined as the presence of an alteration in plasma levels of any lipid and lipoprotein above mentioned. Metabolic syndrome was defined employing Cook' s criteria (Cook et al., 2003) with the exception for glucose levels above 5.55 mmol/l (Genuth et al., 2003; Reinehr et al., 2007).

Data analysis

Data distribution was evaluated for each parameter employing the Shapiro–Wilks test. Variables with normal distribution were described as mean and SD and those distributed in a non parametric way as median and lower and upper quartiles. Spearman's rank correlation rho test was performed to describe the association between physical activity and age. Linear regression models were performed to describe the association between physical activity and each continuous variable. Age and BMI-adjusted partial coefficient of correlation and p values were obtained from these models. Statistical significance was evaluated according to Student's t statistic. For metabolic conditions (categorical variables), logistic regression models (Maximum likelihood; Quasi Newton) were applied and age and BMI-adjusted partial coefficient of correlation and p values were also obtained. Statistical significance was evaluated according to χ^2 distribution. Moreover, stepwise regression analysis was used as discriminant function setting physical activity as the independent variable. Stepwise procedure was performed twice, for continuous and for categorical variables. Variables associated with physical activity at $p < 0.05$ entered in the model. Statistical significance was evaluated according to Student's t statistic or χ^2 distribution. Analyses were carried out using the R statistical software package version 2.9.1 (R Development Core Team, 2009).

Results

A total of 377 male children and adolescents aged 7–14 years were assessed. General clinical and biochemical parameters are shown in Table 1. Based on these data and employing standardized cut-off points and reference values, the presence of different metabolic situations was analyzed in this group of children and adolescents. The most frequent abnormalities detected in the present survey were (n; %) dyslipidemia (93; 28), particularly hypercholesterolemia (60; 18), hypertension (70; 21) and overweight (55; 16)/abdominal obesity (55; 16), followed by hypertriglyceridemia (35; 10), hypoalphalipoproteinemia (15; 4)/hyperglycemia (15; 4), and metabolic syndrome (11; 3).

Different lipoprotein-associated transfer proteins and enzymes, known to modulate the risk of cardiovascular disease, were also determined (Table 2). Among them, PON activity resulted to be the parameter with the greatest dispersion, which may be attributed to the fact that the enzymatic activity evaluated with paraoxon as substrate (PON activity) is sensitive to genetic polymorphism (Q or R alleles), while using phenylacetate as substrate [arylesterase (ARE) activity] is not.

The amount of physical activity was inversely associated with age ($r = -0.30$; $p < 0.001$) and BMI ($r = -0.12$; $p < 0.05$), but not with waist circumference. For this reason, the correlations between physical activity and several clinical and biochemical parameters were performed adjusting by age and BMI: systolic blood pressure (0.14; $p < 0.01$), diastolic blood pressure (0.12; $p < 0.05$), glucose ($r = -0.22$; $p < 0.001$), total cholesterol ($r = -0.16$; $p < 0.005$), and LDL-C ($r = -0.12$; $p < 0.05$). Similarly, physical activity also showed different significant age and BMI-adjusted associations with some metabolic conditions: hypertension ($r = 0.14$; $p < 0.05$), hyperglycemia ($r = -0.19$; $p < 0.05$) and hypercholesterolemia ($r = -0.10$; $p < 0.05$). Furthermore, the practice of physical activity exhibited further negative correlations with CETP ($r = -0.23$, $p < 0.05$) and Lp-PLA$_2$ ($r = -0.39$, $p < 0.001$) activities (Fig. 1).

Stepwise multiple regression analysis was performed in order to identify variables independently associated with physical activity. Age always appeared to be significantly linked to physical activity (Tables 3 and 4). Additionally, Lp-PLA$_2$ activity and the metabolic conditions overweight and hyperglycemia were connected to physical activity in an independent way.

Table 1
General clinical and biochemical parameters from school children (n = 337).

Parameter	Median (1st Q, 3rd Q)
Age (years)	11 (9, 12)
BMI (kg/m^2)	18 (16, 20)
Waist circumference (cm)	64 (59, 70)
SBP (mm Hg)	100 (90, 110)
DBP (mm Hg)	60 (60, 70)
Heart rate (beats/min)	80 (76, 88)
Physical activity (hours/year)	576 (432, 776)
Glucose (mmol/l)	4.77 (4.55, 5.05)
TG (mmol/l)	0.72 (0.57, 0.92)
TC (mmol/l)	4.06 (3.67, 4.52)
LDL-C (mmol/L)	2.21 (1.85, 2.68)
HDL-C (mmol/L)	1.43 (1.27, 1.59)

1st Q, 3rd Q, first and third quartiles; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; TC, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol.

Discussion

The main finding of the present study was that in male children and adolescents' physical activity was inversely associated with different proatherogenic agents following adjustment for age and BMI, being remarkable the negative relationship with CETP and Lp-PLA$_2$ activity. Strikingly, among the parameters evaluated, overweight, hyperglycemia and Lp-PLA$_2$ activity resulted to be independently related to physical activity.

It has been well established that CETP transfers cholesteryl esters from HDL to apo B-containing lipoproteins and triglycerides in the opposite direction. Increased CETP activity leads to the generation of the highly atherogenic small and dense LDL particles and the formation of triglyceride-enriched HDL subfractions, known to be less efficient in their antiatherogenic capacities (Davidson, 2010). In this group of subjects, CETP activity, total cholesterol and triglycerides were inversely associated with exercise, thus revealing higher CVD risk in sedentary individuals. This result agrees with a previous study in which aerobic

Table 2
Activities of lipoprotein-associated transfer proteins and enzymes from school children (n = 87).

Parameter	Median (1st Q, 3rd Q) or mean ± S.D.
CETP (%/ml·h)	228 (216, 246)
PON (nmol/ml·min)	362 (148, 481)
ARE (μmol/ml·min)	128 (110, 150)
Lp-PLA$_2$ (μmol/ml·h)	4.39 ± 1.38

S.D., standard deviation; 1st Q, 3rd Q, first and third quartiles; CETP, cholesteryl ester transfer protein; PON, paraoxonase; ARE, aryleserase; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$.

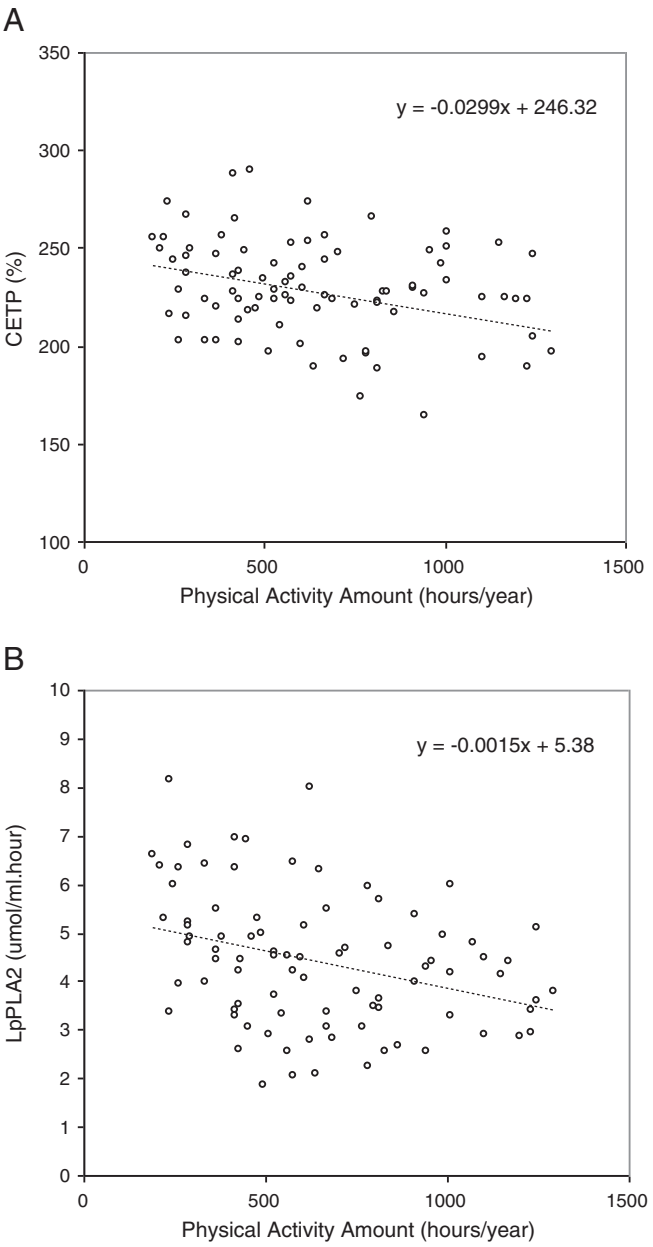

Fig. 1. Variation of CETP (Panel A) and Lp-PLA$_2$ (Panel B) activities with physical activity.

exercise training significantly diminished plasma CETP concentration and improved the lipid profile (Seip et al., 1993).

Lp-PLA$_2$ is an enzyme that degrades oxidatively fragmented phospholipids, which may play a major role in atherogenesis (Tselepis and John Chapman, 2002). Plasma Lp-PLA$_2$ activity is primarily associated with LDL, while a small proportion of the enzyme activity is associated with HDL. Epidemiological studies reported that increased Lp-PLA$_2$ mass or activity was associated with an increased risk of cardiac death, myocardial infarction, acute coronary syndromes and ischemic stroke (Wilensky and Macphee, 2009; Epps and Wilensky, 2011). Therefore, the negative association detected between physical activity and Lp-PLA$_2$ in this group of children and adolescents would suggest a way of blocking the sequence of events leading to plaque formation and rupture present even at young age.

Interestingly, the fact that Lp-PLA$_2$ resulted to be the unique continuous variable that persisted associated with exercise amount after stepwise regression would add an additional benefit of exercise in early prevention of vascular inflammation and atherogenesis. Among the metabolic conditions evaluated in this study, only overweight and hyperglycemia remained independently associated with the amount of physical activity.

Table 3
Stepwise regression analysis between physical activity and different general clinical and biochemical parameters and activities of lipoprotein associated transfer proteins and enzymes from school children (n = 87).

Parameter	Estimate (S.E.)	Pr(>|t|)
Age	−77.77 (13.77)	<0.001
Lp-PLA$_2$	−75.05 (19.01)	<0.001

S.E., standard error; Pr(>|t|), probability associated to Student's t distribution; Lp-PLA$_2$, lipoprotein-associated phospholispase A$_2$. The following variables were included in the analysis: age, body mass index, waist circumference, systolic blood pressure, diastolic blood pressure, heart rate, glucose, triglycerides, total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, cholesteryl ester transfer protein, paraoxonase, aryleserase, paraoxonase 1 phenotype, and Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$.

Taking into account the age of the studied population, it is noteworthy the high frequency of certain metabolic conditions related to increased risk of CVD such as dyslipidemia (28%), particularly hypercholesterolemia (18%), hypertension (21%), overweight (16%) and abdominal obesity (16%). Several factors may contribute to these adverse situations, most likely depending on genetic bases, dietary characteristics and insufficient physical activity.

Accordingly, negative associations were detected between physical activity and different general clinical and biochemical parameters (BMI, glucose, total cholesterol and LDL-C), as well as diverse metabolic conditions (overweight, hyperglycemia and hypercholesterolemia), all of them closely related with high risk of CVD. It may be noted that the magnitudes of the individual biological benefits observed in association with physical activity are not too high, but given that several metabolic conditions are affected at the same time, the global benefit conferred by exercise could be considerable in terms of general health improvement.

Moreover, the results of some observational studies support a dose–response relationship between the amount of physical activity and improvements in insulin sensitivity and glucose control (Haskell, 2001). Exercise duration is one of the primary factors that control the response of insulin action to exercise training. Furthermore, it has been reported that overall adiposity (BMI) predicts insulin resistance (Cummings et al., 2010). Base on the results obtained from the stepwise regression analysis of metabolic abnormalities, it could be concluded that exercise modulates glucose uptake, at least, by means of two mechanisms; one dependent and the other independent on overall adiposity. This situation may reflect the fact that exercise and insulin utilize different signaling pathways to activate glucose transport into the cells (Goodyear and Kahn, 1998).

To get further insights into the mechanisms underlying physical activity benefits, another lipoprotein-associated enzyme was measured: PON 1 (Rosenblat and Aviram, 2009). This enzyme is known to act modulating the oxidative process undergone by lipoproteins in the artery wall. PON 1 is an antioxidant enzyme exclusively bound to HDL and its activity has been shown to be significantly higher in sportsmen than in

Table 4
Stepwise regression analysis between physical activity and different metabolic conditions (n = 337).

	Estimate (S.E.)	Pr(>|Chi sq|)	Odds ratio (CI 95%)
Age	−0.25 (0.065)	<0.001	–
Overweight	−0.69 (0.32)	<0.05	0.28 (0.06, 0.92)
Hyperglycemia	−1.28 (0.67)	<0.05	0.50 (0.27, 0.93)

S.E., standard error; Pr(>|Chi sq|): probability associated to χ^2 distribution.
Physical activity was considered as a binomial variable, defining low and high physical activity upon the median. The following variables were included in the model: age, overweight, abdominal obesity, hypertension, hyperglycemia, hypertriglyceridemia, hypercholesterolemia and hypoalphalipoproteinemia.

sedentary controls (Cakmak et al., 2010). In the present study, PON activity seems to follow the same pattern than its carrier, HDL, which showed no association with the amount of exercise performed.

A limitation of the present study was the observational design by which causality cannot be inferred. However, cross-sectional designs allow evidencing both simple and independent associations and, on the other hand, from an ethical point of view, interventional studies are not always recommended to be performed in children and adolescents. Another limitation deals with the fact that evaluation of physical activity was performed employing a questionnaire and not through a direct method. Nevertheless, we employed a previously validated questionnaire completed in a face to face interview by the same professional who had been previously trained.

In conclusion, in this representative cohort of children and adolescents aged 7–14 years from a rural area of Argentina, physical activity was inversely associated with different proatherogenic agents and situations, being remarkable the negative relationship with overweight, hyperglycemia and the novel biomarker and/or risk factor of inflammation and atherosclerosis, Lp-PLA_2.

Conflict of interest statement

The authors have no affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. All financial and material support for this research and work are clearly identified in the manuscript. The authors have no relevant financial interests in this manuscript.

Acknowledgments

This work was financially supported by “Dr. Felipe A. Fossati” Balcarce Hospital and by Sports and Recreation Subsecretary, Balcarce Council, and grants from University of Buenos Aires (UBACYT B403 and 20020090200017) and from CONICET (PIP 931). We acknowledge the dedicated work and assistance of professionals, laboratory technicians, paramedics, assessors and all other people that were involved in the study. Finally, we specially wish to acknowledge the children and adolescents and their respective parents and teachers that participated in this study.

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