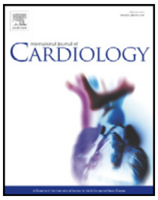
International Journal of Cardiology 186 (2015) 186–195

Contents lists available at ScienceDirect

International Journal of Cardiology

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



The combined effect of physical activity and sedentary behaviors on a clustered cardio-metabolic risk score: The Helena study

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ARTICLE INFO

Article history:
Received 28 November 2014
Received in revised form 9 February 2015
Accepted 16 March 2015
Available online 17 March 2015

Keywords:
Physical activity
Sedentary lifestyle
Cardiometabolic risk
Adolescents

ABSTRACT

Background/objective: Increased physical activity (PA) and decreased sedentary behaviors (SBs) may have beneficial effects on cardio-metabolic risk in adolescents. The aim of this study was to examine the associations between independent/combined effects of PA and SB with individual/clustered cardio-metabolic risk factors.
Methods: A sample of 769 adolescents (12.5–17.5 years) from the HELENA cross-sectional study (Healthy Lifestyle in Europe by Nutrition in Adolescence) and with valid data on metabolic risk factors were included.
Results: Concerning moderate-to-vigorous-PA (MVPA) and vigorous-PA (VPA), measured with accelerometers, girls tended to do more MVPA (36%) and VPA (114%) than boys. Unadjusted analyses show a positive association between "PA $\geq$ 60 min/d; SB $\geq$ 2 h" and the ratio TC/HDL-c ($\beta = 0.27$; 95%CI 0.01 to 0.52; $p < 0.05$), and a negative association between "MVPA $\geq$ 60 min/d; SB < 2 h" with the $\sum$4Skinfolds ($\beta = -0.32$; 95%CI -0.61 to -0.02; $p < 0.05$). Moreover, "SB $\geq$ 2 h/d" was associated with increased cardio-metabolic risk (PR 1.59; 95%CI 1.05 to 2.39; $p < 0.05$), while "PA $\geq$ 60 min/d; SB < 2 h" had a protective effect against cardio-metabolic risk (PR 0.48; 95%CI 0.25 to 0.91; $p < 0.05$). After adjustment for potential confounders, a positive association between SB and $\sum$4Skinfolds was shown ($\beta = 0.28$; 95%CI 0.04 to 0.53; $p < 0.05$). Furthermore, VO_2max (mL/kg/min) tends to increase in those participants who do higher VPA and less SB ($p = 0.042$), and there was a protective effect of "VPA $\geq$ 30 min/d; SB < 2 h" against cardio-metabolic risk (PR 0.24; 95%CI 0.07 to 0.85; $p < 0.05$).
Conclusion: The current study suggests that adolescents should be encouraged to decrease sedentary lifestyle and increase physical activity, especially vigorous physical activity, in order to reduce cardio-metabolic risk.

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http://dx.doi.org/10.1016/j.ijcard.2015.03.176
0167-5273/

1. Introduction

Increased physical activity (PA) and decreased sedentary behaviors (SBs) are important contributors to adolescents' health. On the one hand, SB defined as sitting or lying activities associated with low levels of energy expenditure, has recently been identified as an important contributor to low cardio-metabolic health [1], and adiposity in children [2–4]. On the other hand, PA is an important determinant of obesity and associated comorbidities in young people [5–9].

Several studies concluded that low levels of PA and high levels of SB have been associated with increased overall cardio-metabolic risk [10–13] and overweight [14,15] among children. Some of them have analyzed the associations between PA and single cardiovascular diseases risk factors [13,14,16], and have shown that these associations are often very weak. In this sense, clustering of cardiovascular diseases risk factors has proved to be a better measure of cardiovascular health in children than single risk factors [17,18].

Although, there are studies assessing the independent associations of PA and SB with cardio-metabolic risk factors, little is known about the combined association of PA and SB with individual or clustered cardio-metabolic risk factors [19].

Accurate measurement of these behaviors is important for investigating the associations with health outcomes. Accelerometry is now widely applied for the objective assessment of PA and sedentary time in children [20] with known validity and reliability [21]. Despite the advantages of accelerometers, they have also some short-comings that can be addressed by using survey instruments such as questionnaires. Accelerometers are not able to provide contextual information about children's PA and SB. They do not provide information on the type of PA (e.g. structured vs. unstructured) or specific SB (e.g. television viewing, computer use, painting) and the setting in which it was performed (e.g. at school or at home) [22]. Questionnaires provide a subjective estimate of overall levels of PA and SB during the reference period, as information derived from self-reports is potentially subject to response bias [23]. Nevertheless, improvements in questionnaire design have been thought to ameliorate such limitations.

The aim of this study was to examine the associations between independent and combined effect of PA (measured by accelerometry and by questionnaire) and SB (measured by questionnaire) with individual and clustered cardio-metabolic risk factors in adolescents form the HELENA study.

2. Methods

2.1. Study population

The HELENA study (Healthy Lifestyle in Europe by Nutrition in Adolescence) is a cross-sectional study aiming to describe the lifestyle and nutritional status of European adolescents [24,25]. Data collection took place between 2006 and 2007 in the following cities: Athens and Heraklion (Greece), Dortmund (Germany), Ghent (Belgium), Lille (France), Pecs (Hungary), Rome (Italy), Stockholm (Sweden), Vienna (Austria) and Zaragoza (Spain). A detailed description of the HELENA sampling and recruitment methodology, harmonization processes, data collection, analysis strategies and quality control activities has been published elsewhere [24]. Data were analyzed in August 2014.

A sample of 3528 adolescents met the HELENA general inclusion criteria. One-third of the participating school classes were randomly selected in each city for blood collection, resulting in a total of 1089 adolescents. For the purposes of our study, participants with valid data on cardio-metabolic risk factors (Homeostasis model assessment [HOMA] index, Systolic Blood Pressure [SBP], Triglycerides [TG], total cholesterol/high-density lipoprotein-cholesterol [TC/HDL-c], four skinfold thicknesses [$\sum$4Skinfolds] and maximal oxygen consumption [VO_2max]) were included into the analysis ($n = 769$) (Fig. 1).

The study was performed following the ethical guidelines of the Declaration of Helsinki 1964 as revised in 2000 and was approved by the Ethics Committee of each city involved [24,26]. All parents/guardians signed an informed consent form and the adolescents agreed to participate in the study.

2.2. Individual metabolic risk factors

Anthropometric measurements were measured following a standardized protocol [27]. Weight was measured in underwear and without shoes with an electronic scale (Type SECA 861) to the nearest 0.1 kg; height was measured barefoot in the Frankfort plane with a telescopic height measuring instrument (Type SECA 225) to the nearest 0.1 cm. Waist circumference and a set of skinfold thicknesses (biceps, triceps, subscapular and suprailiac) on the left side of the body were measured three consecutive times with a non-elastic tape (SECA 200) to the nearest 0.1 cm and a Holtain caliper, to the nearest 0.2 mm, respectively.

SBP was measured twice by OMRON®M6 (HEM 70001). Participants were seated in a separate quiet room for 10 min with their backs supported and feet on the ground. Two SPB readings were taken at 10 min intervals, and the lowest measure was used.

Cardiorespiratory fitness was measured using a field-based test (20 m shuttle run test) [28]. Participants ran between two lines spaced 20 m apart, keeping pace with audio signals. The initial speed was 8.5 km/h, and each minute speed was increased by 0.5 km/h. Participants had to run in a straight line and had to pivot on the lines. The test finished when participants stopped due to fatigue or when they failed to reach the end line concurrent with the signals on two consecutive occasions. The last completed stage or half-stage was recorded. Finally, the VO_2max in ml/kg/min was estimated by the Leger equation [28].

Blood samples were collected by venipuncture at school between 8:00 am and 10:00 am after a 10-h overnight fast. Blood was collected in heparinized tubes, immediately placed on dry ice and centrifuged within 30 min (3500 r.p.m. for 15 min) to avoid hemolysis. Immediately after centrifugation, the samples were stored and transported at 4–7 °C (for a maximum of 14 h) to the central laboratory in Bonn (Germany), and stored there at −80 °C until assayed [29]. TG, TC and HDL-c were measured using enzymatic methods (Dade Behring, Schwalbach, Germany). Insulin levels were measured from frozen serum using an Immulite 2000 analyzer (DPC Bierman GmbH, Bad Nauheim, Germany). The HOMA index calculation was used as a measure of insulin resistance [30].

2.3. Clustered cardio-metabolic risk

A clustered cardio-metabolic risk index was created from the following variables: HOMA index, SBP, TG, TC/HDL-c, VO_2max and the $\sum$4Skinfold. The standardized value of each variable was calculated as (value-mean) / standard deviation, separately for males and females and by 1-year age group. For variables characterized by lower metabolic risk with increasing values (HDL-c), z-scores were multiplied by −1. To create the cardio-metabolic risk score, all z-scores were summed, in which the lowest values were indicative of a better cardio-metabolic risk profile. Finally, all of those subjects at or above age- and gender-specific were classified as having metabolic risk when they accumulated $\geq$1SD, similar to previous studies [10,31,32].

2.4. Independent variables

The PA and SB levels were considered independent variables. SB was assessed by questionnaire and PA was assessed both by questionnaire and accelerometry. The questionnaire used for PA measurement was developed to assess PA levels (moderate-to-vigorous levels) in adolescents (IPAQ-A) [33].

PA was also objectively measured with accelerometers (Actigraph MTI, model GT1M, Manufacturing Technology Inc., Fort Walton

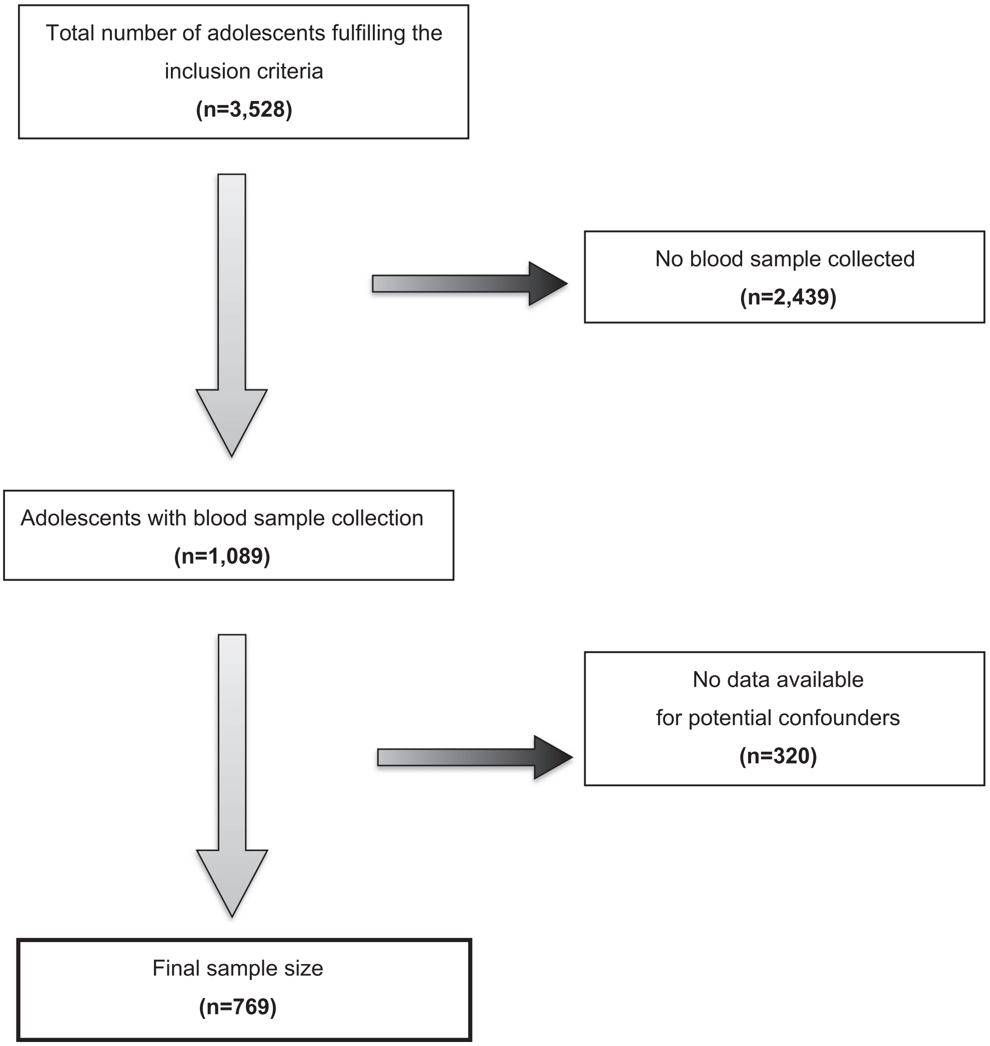

Fig. 1. Final sample size flowchart.

Beach, FL, USA) for seven consecutive days, with a minimum of 8 h recording/day for at least 3 days [34]. The time sampling interval (epoch) was set to 15 s. Inactive, moderate and vigorous PA was defined as <100, 2000–3999 and ≥ 4000 counts/minute, respectively. The cutoffs selected were similar to those used in previous studies [34,35]. The accelerometer is a valid and reliable instrument for measuring PA in adolescents in both the laboratory environment as well as during outdoor activities [36–38]. For both methods (questionnaire and accelerometry), and following current PA guidelines [39–41], participants were classified as active when they accumulated at least 60 min/d of moderate-to-vigorous PA (MVPA) or at least 30 min/d of vigorous PA (VPA) [42].

SB levels were assessed with a structured and reliable questionnaire used in adolescents [43]. This questionnaire includes questions on time habitually spent in front of the television, the computer and/or playing video games [44]. Participants were classified into the following categories, based on the total time spent on SB per day: <2 h/d and ≥ 2 h/d, according to the American Academy of Pediatrics (AAP's) guidelines [45].

We established three clusters combining PA and SB, measured by questionnaires (PA and SB) and accelerometry (MVPA and VPA), as independent variables, which are described below:

- Clustered PA_SB (both measured by questionnaires):
  PA < 60 min/d; SB ≥ 2 h
  PA < 60 min/d; SB < 2 h
  PA ≥ 60 min/d; SB ≥ 2 h
  PA ≥ 60 min/d; SB < 2 h
- Clustered MVPA_SB:
  MVPA < 60 min/d; SB ≥ 2 h
  MVPA < 60 min/d; SB < 2 h
  MVPA ≥ 60 min/d; SB ≥ 2 h
  MVPA ≥ 60 min/d; SB < 2 h
- Clustered VPA_SB:
  VPA < 30 min/d; SB ≥ 2 h
  VPA < 30 min/d; SB < 2 h
  VPA ≥ 30 min/d; SB ≥ 2 h
  VPA ≥ 30 min/d; SB < 2 h

2.5. Potential confounders

The potential confounders for this study were:

- Sex.
- Age (years).
- Center: according to cities participating in The HELENA study.
- Seasonality: corresponds to the date in which accelerometers were programmed to start counting, by three groups: winter, autumn and spring [46].

- Maternal education level: as a marker of the socioeconomic status, determined with a self-reported questionnaire and classified into four levels: elementary education, lower secondary education, upper secondary education and university degree [47].
- Waist circumference: measured at the midpoint between the lowest point of the rib cage and the top of the iliac crest next to skin with a non-elastic measuring tape to the nearest 0.1 cm.
- Height: measured without shoes to the nearest 0.1 cm using a stadiometer (Seca).
- Carbohydrates, proteins, lipids and total energy intake: A self-administered computer-based 24-hour dietary recall, HELENA-DIAT (Dietary Assessment Tool) [48], was used for diet assessment. The software was based on the Young Adolescents' Nutrition Assessment on Computer (YANA-C) software which has been shown as an accurate tool to collect dietary data among adolescents [49]. Adolescents registered all food and drinks consumed during the previous day according to six meal occasions during school time and assisted by fieldworkers. No data regarding Fridays and Saturdays was collected. Adolescents were asked to fill in the HELENA-DIAT twice on non-consecutive days and within a time span of two weeks. The macronutrients and total energy intake were calculated using the German Food Code and Nutrition Date Base (Bundeslebensmittelschlussel, version II.3.1, 2005). The usual food and nutrients intake were estimated by the multiple source method (MSM). The method considers the between- and within-person variability of the dietary data [50]. Carbohydrate, protein and lipid intakes were both estimated in grams per day (g/d) and total energy intake in calories per day (kcal/d).

2.6. Statistical analysis

The descriptive analyses were presented as medians and interquartile ranges (quantitative variables) according to the variable's distribution. A number of participants and percentages are noted for categorical variables.

The associations of the independent variables (PA, SB and the clustered PA_SB, MVPA_SB and VPA_SB) with a) individual cardio-metabolic risk factors, were analyzed using simple and multilevel linear regression models, and standardized regression coefficients (β) adjusted for potential confounders; b) clustered cardio-metabolic risk, were analyzed using multilevel Poisson regression models with robust variance adjustment and a 95%CI calculated for the prevalence ratio (PR), considering two models: unadjusted and adjusted for potential confounders [34].

The context variable used was "Center". p-Values of ≤ 0.20 were adopted in the univariate analysis [51], since they were necessary to include variables in the multivariate analysis and then the hierarchical model method according to the above-mentioned levels. p-Values < 0.05 or those representing >10% modification in the β of any variable already in the model were considered significant.

No interaction for gender and the studied variables was observed ($p > 0.05$). For this reason, we performed the analysis in both sex together. We performed sensitivity analyses in the sample by comparing PA, SB, clustered PA_SB, MVPA_SB, VPA_SB, SBP, VO_2max and the $\sum$4Skinfold among the subjects who had valid data for all variables included in the study, and the whole sample. No significant differences were found for all variables above described, therefore, avoiding selection bias (data not shown).

The multilevel analyses were performed to test the extent to which center as contextual variable mediate the associations between PA, SB and the clustered PA_SB, MVPA_SB and VPA_SB. Stata 12 (Stata Corp., College Station, TX, USA) was used for all statistical calculations.

3. Results

Descriptive characteristics for girls and boys are shown in Table 1. Boys were taller and heavier and had a larger waist circumference than girls. For individual cardio-metabolic risk factors, boys had significantly higher mean values of SBP than girls. Taking into account PA and SB, measured by questionnaires, boys spent more time in sedentary habits than girls; however, measuring with accelerometers, they were more physically active. Girls tend to be more active (PA ≥ 60 min/d) and less sedentary (SB < 2 h) than boys. Moreover, concerning objectively MVPA and VPA, girls tended to do more MVPA (PA ≥ 60 min/d) and VPA (PA ≥ 30 min/d), and to be less sedentary (SB < 2 h) than boys.

The associations between the independent variables (PA, SB, clustered PA_SB, MVPA_SB and VPA_SB) and the individual cardio-metabolic risk factors, without adjustments, are presented in Table 2. There was a positive association between "PA ≥ 60 min/d; SB ≥ 2 h" and the ratio TC/HDL-c, and a negative association between "MVPA ≥ 60 min/d; SB < 2 h" with the $\sum$4Skinfolds. However, the adjusted analysis showed a positive association between SB and the $\sum$4Skinfolds. Moreover, VO_2max (ml/kg/min) tended to increase in those subjects doing more VPA (VPA ≥ 30 min/d) and less SB (SB < 2 h) (Table 3).

Table 4 shows the associations of metabolic risk cluster and the independent variables. Model 1 shows that "SB ≥ 2 h/d" was associated with increased cardio-metabolic risk, and "PA ≥ 60 min/d; SB < 2 h" had a protective effect against cardio-metabolic risk. However, these associations become non-significant after adjustment for potential confounders. Model 2, that includes adjustments, shows the protective effect of "VPA ≥ 30 min/d; SB < 2 h" against cardio-metabolic risk.

4. Discussion

Cardio-metabolic risk factors in children and adolescents have been assessed in many previous studies; however, while most have examined individual or combinations of risk factors [17], few have studied clustering of those factors contributing to the metabolic syndrome. In this study we used a clustered risk score, combining the risk factors related with cardio-metabolic health, in a subsample of 769 European adolescents (12.5–17.5 years) from the HELENA study. The adoption of the metabolic risk cluster appears to be plausible because it is statistically more sensitive and less susceptible to errors than dichotomous approaches [18].

It is widely known that PA and SB are important behaviors for cardio-metabolic health in both children/adolescents [10–13] and adults [52]. To measure these behaviors, questionnaires and accelerometers are the most widely used instruments but both have known limitations. Accelerometers allow the study of activity patterns, and can provide accurate measurements to evaluate the relationship between PA and health outcomes. Self-reported PA data may not accurately reflect activity patterns due to recall bias [53]. However, they are frequently used to estimate levels of PA and SB due to lower cost, suitability for self-administration and noninvasiveness [54]. Therefore, the combination of accelerometers and questionnaires may allow the precise quantification of both the intensity and the duration of children's PA [53].

In this sense, few studies have addressed the question whether PA and SB are related to overall cardio-metabolic risk and body adiposity independently of other health behaviors or individual cardio-metabolic risk factors in youth [55]. In the unadjusted analyses of this study, we observed that higher PA combined with higher SB was positively associated with the TC/HDL-c ratio. This finding may indicate the influence of SB on cardiovascular health despite the PA level. Moreover, higher levels of SB were positively associated with adiposity (as measured by the sum of skinfolds), as shown in the European Youth Heart Study among children and adolescents [55]. In this context, the relationship of SB with overall cardio-metabolic risk was partly

Table 1
Characteristics of the study population.

	Boys (n = 365)	Girls (n = 404)	All (N = 769)
Age (y)	14.5 (12.5–17.5)	14.3 (12.5–17.5)	14.4 (12.5–17.5)
Height (cm)	**169.5 (162.8–176.1)****	162.4 (157.7–166.8)	165 (159.4–171.2)
Weight (kg)	**59 (51.6–67.5)****	55 (49.6–61.6)	56.8 (50.2–64.8)
BMI (kg/m^2)	20.4 (18.6–22.3)	20.9 (18.9–23)	20.6 (18.8–22.8)
Waist circumference (cm)	**71.5 (67.5–76.5)****	68.1 (64.3–74.3)	70 (65.5–75.1)
Socioeconomic status			
Lower education (n,%)	20 (6)	38 (10)	58 (8)
Lower secondary education (n,%)	94 (27)	94 (24)	188 (26)
Higher secondary education (n,%)	99 (29)	123 (32)	222 (30)
Higher education/University degree (n,%)	130 (38)	134 (34)	264 (36)
Physical activity (≥60 min/d; n, %)			
By questionnaires	314 (86)	329 (81)	643 (84)
By accelerometers	**153 (60)*****	74 (26)	227 (42)
Sedentary behavior*			
SB < 2 h/d (n,%)	65 (19)	171 (45)	236 (33)
SB ≥ 2 h/d (n,%)	269 (80)	206 (55)	475 (67)
Clustered PA_SB*			
PA < 60 min/d; SB ≥ 2 h	38 (11)	32 (8)	70 (10)
PA < 60 min/d; SB < 2 h	8 (2)	39 (10)	47 (7)
PA ≥ 60 min/d; SB ≥ 2 h	231 (69)	174 (46)	405 (57)
PA ≥ 60 min/d; SB < 2 h	57 (17)	132 (35)	189 (27)
Clustered MVPA_SB*			
MVPA < 60 min/d; SB ≥ 2 h	36 (92)	39 (114)	38 (206)
MVPA < 60 min/d; SB < 2 h	8 (21)	35 (101)	22 (122)
MVPA ≥ 60 min/d; SB ≥ 2 h	42 (107)	13 (38)	27 (145)
MVPA ≥ 60 min/d; SB < 2 h	13 (33)	12 (36)	13 (69)
Clustered VPA_SB*			
VPA < 30 min/d; SB ≥ 2 h	24 (60)	7 (20)	15 (80)
VPA < 30 min/d; SB < 2 h	8 (21)	3 (9)	5 (30)
VPA ≥ 30 min/d; SB ≥ 2 h	53 (134)	50 (146)	52 (280)
VPA ≥ 30 min/d; SB < 2 h	15 (38)	39 (114)	28 (152)
Cardiometabolic risk (n, %)	57 (16)	62 (15)	119 (15)
Individual cardiometabolic risk factors			
HOMA index	1.9 (1.4–2.8)	2 (1.3–2.8)	2 (1.4–2.8)
Systolic BP (mm Hg)	**123 (114–135)****	116 (110–125)	119.5 (111–129)
TC/HDL-c	2.9 (2.5-–3.3)	2.9 (2.5–3.3)	2.9 (2.5–3.3)
TG (mg/dL)	61 (46–80)	61 (46–83)	61 (46–82)
$\sum$4Skinfolds (mm)	48.2 (31.3–67.5)	48.3 (32.8–66.6)	48.2 (32.1–67)
VO$_2$max (ml/kg/min)	39.6 (32.4–52.2)	40.1 (32.4–53.1)	39.7 (32.4–52.7)

Abbreviations: BMI, body mass index; BP, blood pressure; HOMA, homeostasis model assessment; MVPA, moderate-to-vigorous physical activity; PA, physical activity; SB, sedentary behavior; TC/HDL-c, total cholesterol/High density cholesterol; TG, Triglycerides; VO$_2$max, maximal oxygen consumption; VPA, vigorous physical activity. SB measured by Questionnaires; MVPA and VPA measured by accelerometers. The p-values show sex differences using Pearson product moment correlation coefficient x^2 tests and Mann–Whitney U tests. Statistical significant differences between sexes are depicted as: *p linear tendency < 0.001; **p < 0.01; ***p < 0.001.

accounted for body adiposity and the number of meals [55]. Another study also showed that the direct association of watching TV with body adiposity was partly explained by total PA [56]. Regarding the PA intensity, some cross-sectional studies have examined the association between MVPA and body fat showing an inverse association between these two variables [35,57]. In that sense, our study shows an inverse association between higher MVPA and lower SB with adiposity.

In addition, in the unadjusted analyses we found that higher levels of SB were associated with increased cardio-metabolic risk. On the contrary, as shown in several studies, the time spent in MVPA per day was inversely associated with the metabolic risk score [11,40,52,58,59], indicating that individuals who are more physically active may present lower total metabolic risk scores. After adjusting the analyses for potential confounders, the described associations were not confirmed.

Our adjusted analyses show that the combination of low SB (<2 h/d) with high levels of PA (≥60 min/d) and VPA (≥30 min/d) was related to low cardio-metabolic risk in adolescents. There are a number of studies on the association of PA and SB with overall cardio-metabolic risk among children [10–15,60]. Thus, decreasing SB, increasing PA and avoiding unhealthy eating may reduce cardio-metabolic risk among children and adolescents [56]. The vigorous PA increases the efficiency of the left ventricular mass and volumes (diastolic and systolic volumes and ejection fraction) [61], these changes are considered favorable to have healthy metabolic conditions [62].

Finally, the adolescents who have a higher VPA habit combined with a low SB have a higher cardiorespiratory fitness. Likewise, a recent study suggests that those adolescents categorized as active/low sedentary were more likely to have a higher overall score of physical fitness than those categorized as inactive/high sedentary [63]. Therefore, children and adolescent's physical fitness may influence the possible effect of PA on various health parameters. In this sense, several studies have shown, in this population, an association between increased aerobic capacity and lower levels of total adiposity [64,65], truncal fat [66] and waist circumference [67,68]. Moreover, few studies show that high levels of physical fitness are associated with improved health-related biomarkers and may further influence health in young adulthood [69–72]. These findings highlight the importance of increasing PA and decreasing time devoted to SB in improving young people's health and possibly prevent cardiometabolic diseases [73].

A limitation of this study is its cross-sectional design; hence temporal sequence cannot be established and rules out the possibility to draw causal conclusions. Another limitation is that VO$_2$max was estimated and this can lead to a less accurate data. However, our results come from the clustered PA and SB, and that may be the reason for a weak association. Besides these limitations, our study has some strengths such as the diverse geographic origin of the samples, its harmonized and standardized methodology to concurrently assess both objective and subjective measures of PA and SB, and the multilevel adjusted analysis.

Table 2
Unadjusted simple linear regression analysis: association between cardiometabolic risk cluster components according to independent variables.

	HOMA index β (95% CI)	Systolic BP (mm Hg) β (95% CI)	TC/HDL-c β (95% CI)	TG (mg/dL) β (95% CI)	∑ 4Skinfolds (mm) β (95% CI)	VO_2max (mL/kg/min) β (95% CI)
Physical activity						
[By questionnaires]						
PA ≥ 60 min/d	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
PA < 60 min/d	−0.07 (−0.27 to 0.13)	0.07 (−0.12 to 0.26)	−0.14 (−0.34 to 0.05)	0.05 (−0.14 to 0.23)	−0.13 (−0.33 to 0.08)	−0.04 (−0.24 to 0.16)
Physical activity						
[By accelerometers]						
PA ≥ 60 min/d	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
PA < 60 min/d	0.05 (−0.12 to 0.23)	−0.01 (−0.17 to 0.16)	0.13 (−0.04 to 0.30)	0.05 (−0.12 to 0.23)	0.13 (−0.06 to 0.31)	−0.02 (−0.20 to 0.16)
Sedentary behavior						
SB < 2 h/d	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
SB ≥ 2 h/d	0.11 (−0.05 to 0.28)	0.09 (−0.07 to 0.24)	0.09 (−0.07 to 0.24)	0.10 (−0.05 to 0.25)	0.13 (−0.04 to 0.29)	−0.15 (−0.31 to 0.02)
Clustered PA_SB						
PA < 60 min/d; SB ≥ 2 h	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
PA < 60 min/d; SB < 2 h	0.09 (−0.29 to 0.48)	−0.15 (−0.52 to 0.22)	0.13 (−0.24 to 0.50)	−0.06 (−0.42 to 0.30)	0.02 (−0.37 to 0.42)	−0.04 (.0.43 to 0.35)
PA ≥ 60 min/d; SB ≥ 2 h	0.12 (−0.15 to 0.38)	−0.07 (−0.33 to 0.19)	**0.27 (0.01 to 0.52)***	−0.06 (−0.31 to 0.18)	0.19 (−0.08 to 0.46)	−0.01 (−0.28 to 0.25)
PA ≥ 60 min/d; SB < 2 h	−0.04 (−0.33 to 0.24)	−0.15 (−0.43 to 0.13)	0.14 (−013 to 0.42)	−0.18 (−0.45 to 0.09)	0.04 (−0.25 to 0.33)	0.18 (−0.11 to 0.47)
Clustered MVPA_SB						
MVPA < 60 min/d; SB ≥ 2 h	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
MVPA < 60 min/d; SB < 2 h	−0.13 (−0.36 to 0.10)	0.001 (−0.21 to 0.22)	−0.11 (−0.34 to 0.11)	−0.11 (−0.34 to 0.12)	−0.22 (−0.46 to 0.03)	0.12 (−0.12 to 0.36)
MVPA ≥ 60 min/d; SB ≥ 2 h	−0.11 (−0.33 to 0.11)	−0.04 (−0.24 to 0.17)	−0.21 (−0.42 to 0.01)	−0.14 (−0.35 to 0.08)	−0.20 (−0.43 to 0.03)	−0.01 (−0.24 to 0.22)
MVPA ≥ 60 min/d; SB < 2 h	−0.09 (−0.37 to 0.19)	0.01 (−0.25 to 0.28)	−0.17 (−0.45 to 0.12)	−0.08 (−0.36 to 0.19)	**−0.32 (−0.61 to −0.02)***	0.08 (−0.21 to 0.38)
Clustered VPA_SB						
VPA < 30 min/d; SB ≥ 2 h	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
VPA < 30 min/d; SB < 2 h	−0.20 (−0.63 to 0.23)	−0.36 (−0.77 to 0.05)	0.31 (−0.12 to 0.73)	−0.002 (−0.43 to 0.42)	0.05 (−0.41 to 0.51)	−0.13 (−0.58 to 0.31)
VPA ≥ 30 min/d; SB ≥ 2 h	0.01 (−0.25 to 0.26)	−0.16 (−0.41 to 0.08)	**0.01 (−0.24 to 0.27)****	−0.02 (−0.28 to 0.23)	0.05 (−0.22 to 0.32)	−0.19 (−0.45 to 0.08)
VPA ≥ 30 min/d; SB < 2 h	−0.06 (−0.34 to 0.22)	−0.13 (−0.39 to 0.14)	−0.08 (−0.35 to 0.20)	−0.04 (−0.31 to 0.23)	−0.14 (−0.43 to 0.16)	0.01 (−0.28 to 0.30)

Abbreviations: BP, blood pressure; HOMA, homeostasis model assessment; MVPA, moderate-to-vigorous physical activity; PA, physical activity; SB, sedentary behavior; TC/HDL-c, total cholesterol/high density cholesterol; TG, Triglycerides; VO_2max, maximal oxygen consumption; VPA, vigorous physical activity. SB measured by questionnaires; MVPA and VPA measured by accelerometers. Standardized regression coefficients (β) are from simple linear regression.

* $p < 0.05$.

** $p < 0.01$.

Table 3

Multilevel analysis: association between cardiometabolic risk cluster components according to independent variables (adjusted model).

	HOMA index	Systolic BP (mm Hg)	TC/HDL-c[a]	TG (mg/dL)	$\sum$ 4Skinfolds (mm)	VO$_2$max (mL/kg/min)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Physical activity	p = 0.06	p = 0.86	p = 0.35	p = 0.43	p = 0.89	p = 0.65
[By questionnaires]	0.26 (−0.01 to 0.53)	−0.23 (−2.85 to 2.38)	−0.15 (−0.48 to 0.17)	0.08 (−0.12 to 0.28)	−0.02 (−0.34 to 0.30)	−0.08 (−0.41 to 0.26)
Physical activity	p = 0.48	p = 0.58	p = 0.53	p = 0.17	p = 0.493	p = 0.25
[By accelerometers]	0.08 (−0.14 to 0.29)	−0.65 (−2.94 to 1.64)	0.11 (−0.22 to 0.44)	0.12 (−0.05 to 0.30)	−0.10 (−0.38 to 0.18)	0.17 (−0.12 to 0.45)
Sedentary behavior (SB)	p = 0.65	p = 0.79	p = 0.19	p = 0.36	**p = 0.02**[*]	p = 0.15
	0.05 (−0.16 to 0.25)	0.28 (−1.80 to 2.37)	0.18 (−0.09 to 0.46)	0.07 (−0.08 to 0.23)	**0.28 (0.04 to 0.53)**	−0.19 (−0.45 to 0.07)
Clustered PA_SB	p = 0.07	p = 0.99	p = 0.86	p = 0.27	p = 0.26	p = 0.24
PA < 60 min/d; SB ≥ 2 h	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
PA < 60 min/d; SB < 2 h	0.35 (−0.13 to 0.82)	−1.20 (−5.93 to 3.52)	−0.27 (−0.88 to 0.34)	0.04 (−0.32 to 0.40)	0.03 (−0.56 to 0.63)	−0.38 (−1.00 to 0.25)
PA ≥ 60 min/d; SB ≥ 2 h	−0.07 (−0.41 to 0.27)	−0.21 (−3.51 to 3.10)	0.11 (−0.29 to 0.52)	−0.03 (−0.28 to 0.23)	0.15 (−0.24 to 0.55)	−0.16 (−0.57 to 0.25)
PA ≥ 60 min/d; SB < 2 h	−0.20 (−0.56 to 0.16)	−0.28 (−3.88 to 3.32)	−0.05 (−0.50 to 0.40)	−0.13 (−0.40 to 0.15)	−0.19 (−0.61 to 0.22)	0.15 (−0.29 to 0.59)
Clustered MVPA_SB	p = 0.25	p = 0.81	p = 0.34	p = 0.09	p = 0.72	p = 0.78
MVPA < 60 min/d; SB ≥ 2 h	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
MVPA < 60 min/d; SB < 2 h	−0.07 (−0.35 to 0.20)	0.16 (−2.73 to 3.05)	−0.28 (−0.66 to 0.11)	−0.10 (−0.33 to 0.14)	−0.27 (−0.64 to 0.09)	0.17 (−0.20 to 0.53)
MVPA ≥ 60 min/d; SB ≥ 2 h	−0.05 (−0.32 to 0.21)	0.04 (−2.68 to 2.75)	−0.19 (−0.59 to o.20)	−0.17 (−0.39 to 0.05)	0.15 (−0.20 to 0.50)	−0.15 (−0.50 to 0.20)
MVPA ≥ 60 min/d; SB < 2 h	−0.24 (−0.58 to 0.10)	0.56 (−2.83 to 3.94)	−0.16 (−0.66 to 0.34)	−0.18 (−0.47 to 0.10)	−0.32 (−0.78 to 0.13)	0.09 (−0.37 to 0.55)
Clustered VPA_SB	p = 0.96	p = 0.73	p = 0.38	p = 0.94	p = 0.11	**p = 0.04**[*]
VPA < 30 min/d; SB ≥ 2 h	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
VPA < 30 min/d; SB < 2 h	0.01 (−0.50 to 0.54)	−3.55 (−8.74 to 1.64)	0.31 (−0.47 to 1.08)	0.03 (−0.41 to 0.47)	−0.75 (−1.43 to −0.06)	−0.16 (−0.85 to 0.53)
VPA ≥ 30 min/d; SB ≥ 2 h	0.08 (−0.23 to 0.40)	−1.86 (−5.02 to 1.30)	0.07 (−0.37 to 0.51)	0.04 (−0.22 to 0.30)	−0.20 (−0.61 to 0.21)	0.14 (−0.27 to 0.56)
VPA ≥ 30 min/d; SB < 2 h	−0.01 (−0.36 to 0.33)	−0.96 (−4.54 to 2.61)	−0.20 (−0.71 to o.31)	0.01 (−0.27 to 0.29)	**−0.48 (−0.93 to −0.03)**	0.40 (−0.05 to 0.85)

Abbreviations: BP, blood pressure; HOMA, homeostasis model assessment; MVPA, moderate-to-vigorous physical activity; PA, physical activity; SB, sedentary behavior; TC/HDL-c, total cholesterol/high density cholesterol; TG, Triglycerides; VO$_2$max, maximal oxygen consumption; VPA, vigorous physical activity. SB measured by questionnaires; MVPA and VPA measured by accelerometers. Standardized regression coefficients (β) are from multilevel linear regression model adjusted for potential confounders.

[*] $p < 0.05$.

[a] Multiple linear regression analysis.

Table 4
Associations of clustered cardiometabolic risk with independent variables. Unadjusted and adjusted multilevel linear regression analysis.

Clustered cardiometabolic risk	PR (95% CI) Unadjusted model	PR (95% CI) Adjusted#
Physical activity		
[By questionnaires]		
PA ≥ 60 min/d	1.00	1.00
PA < 60 min/d	1.16 (0.76 to 1.76)	1.54 (0.77 to 3.08)
Physical activity		
[By accelerometers]		
PA ≥ 60 min/d	1.00	1.00
PA < 60 min/d	1.27 (0.83 to 1.93)	1.18 (0.56 to 2.25)
Sedentary behavior		
SB < 2 h/d	1.00	1.00
SB ≥ 2 h/d	**1.59 (1.05 to 2.39)****	1.96 (0.96 to 4.01)
Clustered PA_SB*		
PA < 60 min/d; SB ≥ 2 h	1.00	1.00
PA < 60 min/d; SB < 2 h	0.85 (0.39 to 1.87)	0.92 (0.26 to 3.20)
PA ≥ 60 min/d; SB ≥ 2 h	0.85 (0.51 to 1.43)	0.74 (0.32 to 1.72)
PA ≥ 60 min/d; SB < 2 h	**0.48 (0.25 to 0.91)****	0.29 (0.09 to 0.88)
Clustered MVPA_SB		
MVPA < 60 min/d; SB ≥ 2 h	1.00	1.00
MVPA < 60 min/d; SB < 2 h	0.61 (0.34 to 1.07)	0.41 (0.14 to 1.25)
MVPA ≥ 60 min/d; SB ≥ 2 h	0.76 (0.47 to 1.24)	0.92 (0.41 to 2.07)
MVPA ≥ 60 min/d; SB < 2 h	0.46 (0.20 to 1.04)	0.21 (0.03 to 1.59)
Clustered VPA_SB		
VPA < 30 min/d; SB ≥ 2 h	1.00	1.00
VPA < 30 min/d; SB < 2 h	0.61 (0.19 to 2.01)	2.26 (0.51 to 5.13)
VPA ≥ 30 min/d; SB ≥ 2 h	1.05 (0.60 to 1.85)	0.63 (0.26 to 1.53)
VPA ≥°30 min/d; SB < 2 h	0.65 (0.33 to 1.28)	**0.24 (0.07 to 0.85)****

Abbreviations: PA, physical activity; SB, sedentary behavior; MVPA, moderate-to-vigorous physical activity; VPA, vigorous physical activity. SB measured by questionnaires; MVPA and VPA measured by accelerometers. Poisson distribution model was used (prevalence ration [PR] and 95% confidence intervals [CI]). #This analysis was adjusted for potential confounders: seasonality, maternal education level, sex, age, waist circumference, height and carbohydrates, proteins, lipids and total energy intake.

* p linear tendency < 0.05.

** p < 0.05.

5. Conclusions

The findings of present study suggest that in order to reduce cardiometabolic risk, adolescents should be encouraged to decrease sedentary lifestyle and increase physical activity, especially vigorous physical activity.

Abbreviations

$\sum$4Skinfolds	Four skinfold thicknesses
HELENA study	Healthy Lifestyle in Europe by Nutrition in Adolescence study
HOMA index	Homeostasis model assessment
VO$_2$max	Maximal oxygen consumption
MVPA	Moderate-to-vigorous physical activity
PA	Physical activity
SB	Sedentary behaviors
SBP	Systolic Blood Pressure
TC/HDL-c	Total cholesterol/high-density lipoprotein-cholesterol
VPA	Vigorous physical activity

Funding and conflicts

The HELENA study takes place with the financial support of the European Community Sixth RTD Framework Programme (contract FOOD-CT-2005-007034). This study was also supported by a grant from the Spanish Ministry of Health: Maternal, Child Health and Development Network, (number RD08/0072), grant from the Spanish Ministry of Education (EX-2008-0641) and the Swedish Heart-Lung Foundation (20090635). Also supported by grants from the Spanish Ministry of Science and Innovation (JCI-2010-07055; RYC-2010-05957).

The author Tara Rendo-Urteaga was given post-doctoral scholarship from Brazilian government by Science without Borders Program by CAPES (Coordination of Improvement of Higher Education Personnel) (proc. 007/2012), and Luis A. Moreno was given a visiting professor scholarship by CNPq (National Counsel of Technological and Scientific Development). Augusto César Ferreira de Moraes was given scholarship from São Paulo Research Foundation — FAPESP (proc. 2011/11137-1 and 2011/20662-2). and receipt a Post-Doctoral scholarship from National Counsel of Technological and Scientific Development (CNPq: proc. 313772/2014-2). The GENUD Research Group co-financed by the European Regional Development Fund (MICINN-FEDER).

The authors have no conflict to disclose and they certify that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

Acknowledgments

We gratefully acknowledge all participating adolescents and their parents for their collaboration, and Petra Pickert and Anke Carstensen for their contribution to laboratory work.

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