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CLINICAL RESEARCH ARTICLE

Daily steps, cardiorespiratory fitness, and remnant cholesterol in schoolchildren: mediation effects for cardiovascular prevention

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BACKGROUND: To analyse the associations between daily steps, cardiorespiratory fitness (CRF), and remnant cholesterol in schoolchildren and to investigate whether the association between daily steps and remnant cholesterol is mediated by CRF.
METHODS: This cross-sectional study involved 394 schoolchildren (aged 9–12 years, 53.0% girls) from Cuenca, Spain. Daily steps were measured using the Xiaomi MI Band 3, CRF was assessed using the 20-m shuttle run test, and remnant cholesterol was calculated from total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. Mean differences in CRF and remnant cholesterol by daily steps and CRF categories were tested using analysis of covariance. Mediation analysis models examined whether CRF mediates the association between daily steps and remnant cholesterol.
RESULTS: Children taking 12,000 and 9000 steps/day had higher CRF ($p < 0.001$) and lower remnant cholesterol ($p = 0.034$), respectively. Those with CRF > 47.59 kg/ml/min had lower remnant cholesterol ($p = 0.009$). CRF mediated the association between 1000 steps/day and remnant cholesterol (indirect effect = –0.027 (–0.055,–0.007)).
CONCLUSIONS: Both daily steps and CRF are associated with remnant cholesterol. Promoting an increase in daily steps may be a practical and promising strategy to increase CRF and, given its mediating role, to improve remnant cholesterol to prevent cardiometabolic risk in schoolchildren.

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IMPACT:

- What's known: Remnant cholesterol is a critical indicator of cardiovascular disease risk in the early atherosclerosis.
- What's new: In schoolchildren, increased daily physical activity is significantly associated with higher cardiorespiratory fitness and lower remnant cholesterol, especially walking >9000 steps/day and >12,000 steps/day, respectively.
- What's relevant: Encouraging schoolchildren to take more daily steps may be a promising strategy to increase cardiorespiratory fitness and, given its mediating role, to improve remnant cholesterol to prevent cardiometabolic risk.

INTRODUCTION

The association between cardiovascular disease (CVD) risk factors in adulthood and cardiovascular events is well documented, suggesting that prevention focused on these cardiovascular risk factors is a public health priority. Atherosclerosis is the root cause of most CVDs in women and men worldwide.[1] Autopsy studies have shown that the atherogenic process can begin early in life,[2] even during foetal development.[3] Maternal hypercholesterolaemia has been shown to increase the risk of atherosclerotic lesions in the foetus and to accelerate the progression of these lesions after birth.[4] In addition to dyslipidemia, modifiable risk factors for atherosclerosis in childhood include hypertension, passive or active exposure to tobacco, type 1 and type 2 diabetes, and obesity.[5]

Consistent evidence supports that exposure to atherosclerotic risk factors in infancy is associated with preclinical atherosclerosis in adulthood but also with cardiovascular events[5] and deaths from CVD before 60 years of age.[6] In parallel, studies have proven that both physical activity-related behaviour and cardiorespiratory fitness (CRF) levels in infancy tend to persist through adolescence

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to adulthood.[7–9] Moreover, these studies have demonstrated that behaviour related to physical activity during infancy is associated with cardiovascular events and mortality in adult life.

Therefore, as evidence suggests that CVD originates in early life, reducing risk factors from childhood to adulthood could decrease the incidence of premature CVD.[6] Among the risk factors involved in CVD risk, two key components have gained significant attention: lipid metabolism[10] and physical activity.[11]

In the context of lipid metabolism, traditional markers such as low-density lipoprotein cholesterol (LDL-C) have been used for cardiovascular risk assessment because of their key role in the pathophysiology of atherosclerosis.[12] However, emerging evidence has highlighted the role of remnant cholesterol as a critical CVD risk indicator in the early stages of atherosclerosis, even in individuals with normal LDL-C levels.[13] Remnant cholesterol consists of very-low-density lipoproteins (VLDLs), intermediate-density lipoproteins, and chylomicron remnants, which contain high levels of cholesterol.[14] Among these, VLDLs play a pivotal role in the transportation of triglycerides from the liver to peripheral tissues. However, when VLDLs are not adequately removed from the bloodstream, they can undergo transformation into smaller, denser, cholesterol-rich remnant particles.[15] VLDLs, together with the other remnant particles, can easily penetrate the arterial wall, resulting in the retention of these particles within the intimal space of the arteries. This process promotes the formation of foam cells and triggers an inflammatory response,[14] which play causal roles in the development of atherosclerosis in adulthood, irrespective of LDL-C.[16]

Conversely, lack of physical activity, together with poor CRF, is an important modifiable risk factor for the primary prevention of CVDs.[17] Hence, the importance of increasing physical activity levels as a public health intervention has been highlighted,[18] as ~80% of children worldwide fail to meet World Health Organisation movement behaviour recommendations.[19] Daily step counting using wearable devices is one of the most effective strategies for increasing physical activity in children.[20] This increase in physical activity may partially contribute to the increase in CRF,[21,22] since 50% of its level is attributed to genetic factors.[23] Furthermore, this relationship does not appear to be linear.[24]

This has important implications for cardiovascular health. In this sense, recent meta-analyses have revealed inverse associations between daily steps and the risk of premature cardiovascular mortality and cardiovascular events in middle-aged and older adults.[25–29] Such an association may be explained, at least partially, by the well-documented fact that physical activity and CRF are associated with favourable traditional lipid and lipoprotein profiles in both children[30,31] and adults.[32,33] However, the associations of physical activity, particularly daily steps, with remnant cholesterol, an independent risk factor for CVD,[34] have not been investigated.

Therefore, the objectives of our study were (1) to analyse the associations between daily steps, CRF, and lipid profiles, including remnant cholesterol, in schoolchildren and (2) to examine whether the association between daily steps and remnant cholesterol is mediated by CRF levels.

METHODS

Study design and participants

Following the STROBE guidelines (Table S1), we performed a cross-sectional analysis of baseline data (collected between February and November 2022) from the e-MOVI study, which aimed to assess physical activity and dietary behaviour, lifestyle, and cardiovascular risk that occur during childhood.

We invited 1288 children aged 9 to 12 years old, belonging to 4th, 5th or 6th grade, from 8 public primary schools in the province of Cuenca, Spain, to participate in the research project if they met the following inclusion criteria: (1) had literacy in Spanish (or Spanish sign language); (2) did not have significant learning difficulties or physical or mental conditions, as identified by parents and teachers, that might prevent participation in e-MOVI; (3) had no allergies to the materials used in the smart wristband that would prevent them from wearing it; and (4) had consent from their parents or legal guardians to participate in the research project. A total of 904 (70.2%) schoolchildren were ultimately enroled in the study. Of these, 510 (39.6%) were removed because of missing data on daily step count, remnant cholesterol or CRF. As a result, the present analyses were performed with 394 children (30.6%) with complete data (Fig. 1).

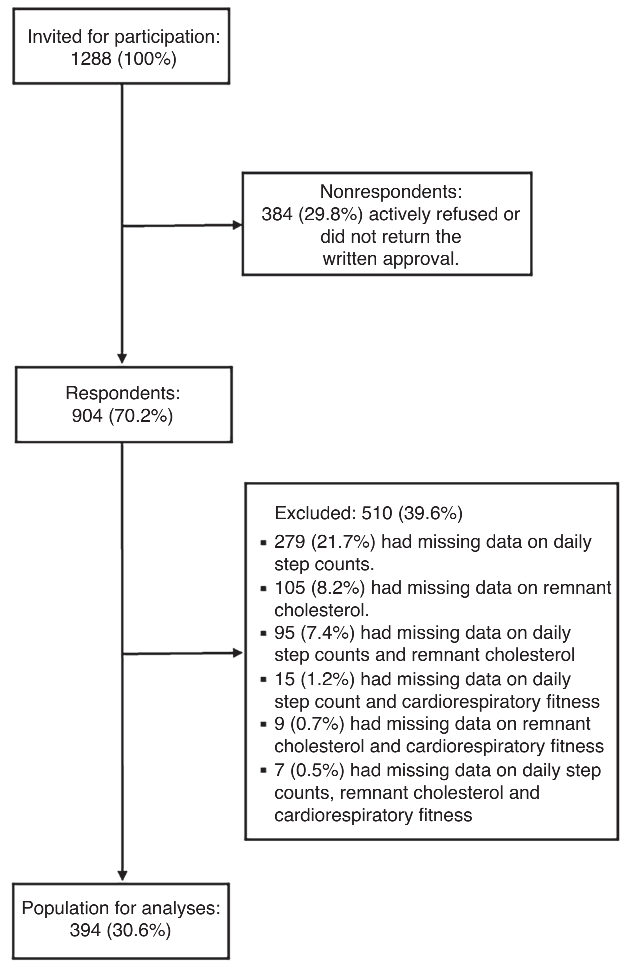

Fig. 1 Flow chart of the study participants in the current study from the original e-MOVI study.

The study protocol received approval from the Clinical Research Ethics Committee of the Hospital Virgen de la Luz de Cuenca (REG: 2019/PI1519). Once the study was approved by the governing board of each school, a letter was sent to the parents of all the students in each grade, inviting them to a meeting. At this meeting, the objectives of the study were explained, and written consent was obtained for their children's participation. Moreover, schoolchildren were informed of the characteristics of the study, and they provided consent to participate. All procedures conducted in this study complied with the Declaration of Helsinki, including its later amendments, or with comparable ethical standards for research involving human participants.[35]

Study variables

The study variables were measured by trained researchers following standardised conditions.

Exposure: daily step count. Participants wore a Xiaomi Mi Band 3 Smart Bracelet on the wrist of the nondominant hand to measure daily steps, which has good validity for daily step counts.[36] The mean of the daily step counts over the 2 weeks following the initial baseline measurements was calculated. Only data from participants whose wristbands had records for

Table 1. Characteristics of the total study sample, and by sex.

	Total (n = 394)	**Boys (n = 185)**	**Girls (n = 209)**	***p* value**
Anthropometry				
Age (years)	9.90 (1.23)	9.87 (1.24)	9.91 (1.23)	0.715
Weight (kg)	39.92 (9.71)	39.72 (9.39)	40.10 (10.00)	0.666
Height (cm)	144.36 (8.36)	143.91 (7.84)	144.76 (8.79)	0.253
BMI (kg/m^2)	18.98 (3.54)	19.02 (3.51)	18.96 (3.57)	0.849
Physical activity/Cardiorespiratory fitness				
Daily steps	10,782 (3258)	12,194 (3216)	9520 (2668)	**<0.001**
VO2 max (ml/kg/min)	46.19 (4.60)	47.53 (5.07)	44.98 (3.74)	**<0.001**
Lipid parameters				
Total cholesterol (mg/dl)	159.40 (27.70)	158.02 (27.75)	160.81 (27.71)	0.318
LDL-c (mg/dl)	83.68 (23.91)	82.14 (23.07)	85.04 (24.60)	0.227
HDL-c (mg/dl)	61.52 (13.25)	62.18 (12.85)	60.80 (13.70)	0.301
Remnant cholesterol (mg/dl)	14.17 (3.49)	13.33 (3.24)	14.92 (3.55)	**<0.001**
Very low-density lipoprotein (mg/dl)	12.05 (4.93)	10.86 (4.31)	13.11 (5.21)	**<0.001**
Triglycerides (mg/dl)	60.27 (24.66)	54.29 (21.56)	65.57 (26.03)	**<0.001**

VO2 max (ml/kg/min) values calculated with the Leger formula (28). The values in bold indicate statistical significance at $p < 0.05$. *P* values are from parametric test as all variables are normally distributed.
BMI body mass index, *VO2 max* maximal oxygen intake, *LDL-c* low-density lipoprotein cholesterol, *HDL-c* high-density lipoprotein cholesterol.

at least 4 days per week, with at least 1 weekend day, were considered. The children recorded their step counts in a daily log, which was collected weekly by a member of the research team at the school.

Outcome: estimated remnant cholesterol. Blood samples were drawn at school from the cubital vein between 8.15 and 9.00 am and after a fasting period of at least 12 h. These determinations were performed in duplicate and included total cholesterol, high-density lipoprotein cholesterol (HDL-C), LDL-C, and triglyceride levels analysed with a Cobas c701 system from Roche Diagnostics. These parameters were recorded in mg/dL.

LDL-c levels were estimated using the Martin–Hopkins equation rather than the Friedewald equation since the latter tends to provide lower estimates of LDL-C when hypertriglyceridaemia (≥150 mg/dl) is present.[37] Therefore, this lipid fraction was determined on the basis of non-HDL-C and triglyceride levels.[38] In addition, based on this equation, VLDL was calculated as triglycerides divided by 5.[38]

Remnant cholesterol levels was calculated as follows: total cholesterol–(HDL-C + calculated LDL-C).

Mediator: estimated cardiorespiratory fitness. The 20-m shuttle run test was used to estimate CRF.[39] The participants were required to run back and fordward between two lines, situated at a distance of 20 m apart, in accordance with the pace set by audio signals emitted from a prerecorded compact disc. The initial speed was 8.5 km/h, increasing gradually by 0.5 km/h each minute. The children were encouraged to continue running as long as they could during the test. When the children failed to maintain their pace for two consecutive intervals, they were asked to stop, and the stage completed (full laps) was recorded. Leger's formula[40] validated for estimating maximal oxygen consumption[41] was used.

Covariates. Age and sex were gathered from school records. Height and body weight were each measured twice and averaged for analysis, with the children dressed in light clothing and barefoot. Height was measured to the nearest mm using a wall-mounted stadiometer (SECA 222, Vogel and Halke, Hamburg, Germany), with the children standing upright with the sagittal midline touching the backboard. Body weight was measured to the nearest 100 g using a calibrated digital scale (SECA 861; Vogel & Halke, Hamburg, Germany). Body mass index (BMI) was calculated by dividing body weight (in kg) by height (in m^2).

To determine the minimum sufficient adjustment set (MSAS) for assessing the total effect of daily step count on remnant cholesterol, we developed a theoretical causal diagram based on previous knowledge available in the scientific literature. We used the online tool DAGitty[42] to construct a directed acyclic graph (DAG). The covariates sex, age, and BMI were identified as the MSAS.

Statistical analysis

The normality of all continuous variables was evaluated using both statistical (Kolmogorov–Smirnov test) and graphical methods (normal probability plots). We then used Student's *t* test to describe the characteristics of the study sample by sex. We generated scatterplots and estimated bivariate correlation coefficients to examine the relationships between daily steps and CRF, BMI, remnant cholesterol, LDL-C, HDL-C, and triglycerides, and between VLDL and remnant cholesterol, daily steps, and CRF.

The mean differences in CRF were analysed using analysis of covariance (ANCOVA) models, with daily step category/tertiles as the dependent variable (rounded to 1000 steps/day, <9000, 9000–12,000, and >12,000) -model 1-, controlling for age, sex, and BMI -model 2-, and adding CRF -model 3- (for remnant cholesterol as the dependent variable). Additionally, we analysed the mean differences in remnant cholesterol according to CRF category/tertiles (<43.46 kg/ml/min, 43.46–47.59 kg/ml/min, and >47.59 kg/ml/min) -model 1-, controlling for age, sex, and BMI -model 2-, and adding daily steps -model 3-.

We performed a simple mediation analysis to examine whether the association between 1000 steps/day increment and remnant cholesterol was mediated by CRF, controlling for age, sex, and BMI (i.e. the MSAS covariates identified through the DAG). For these estimations, we used macro-PROCESS for SPSS version 4.1, selecting model 4 and 5000 bias-corrected bootstrap samples,[43] according to the checklist "A Guideline for Reporting Mediation Analyses" for reporting mediation analyses of observational studies (Table S2). This analysis aimed to examine the total (c) and direct effects (a, b, and c'), representing the unstandardised regression coefficient for every 1000 steps/day increment (independent variable) on remnant cholesterol (dependent variable), as well as the indirect effects that quantify the change in remnant cholesterol for every 1000 steps/day increment mediated by the potential mediator variable (CRF). In the mediation analysis models, *path a* represents the regression coefficients of CRF on a 1000 step/day increment, *path b* represents the regression coefficients of remnant cholesterol on CRF, and the c coefficient (total effect) represents the regression of remnant cholesterol on 1000 steps/day increment. *Path c′* (direct effect) indicates the regression coefficient of remnant cholesterol on 1000 steps/day increment after adjustment for the mediating variable (CRF). In accordance with Hayes' recommendation,[44] this study did not employ the concepts of complete or partial mediation. As illustrated in Fig. S1, when adjusting the analysis for MSAS covariates, the bias pathways were fully closed, leaving only the causal pathways (both direct and indirect, i.e. through mediators) open.

Sensitivity analyses were conducted using multiple imputation of data for daily step counts, remnant cholesterol and CRF to reduce possible bias caused by missing data and maximise the use of available information to preserve statistical power. Multiple imputation is an approach used to

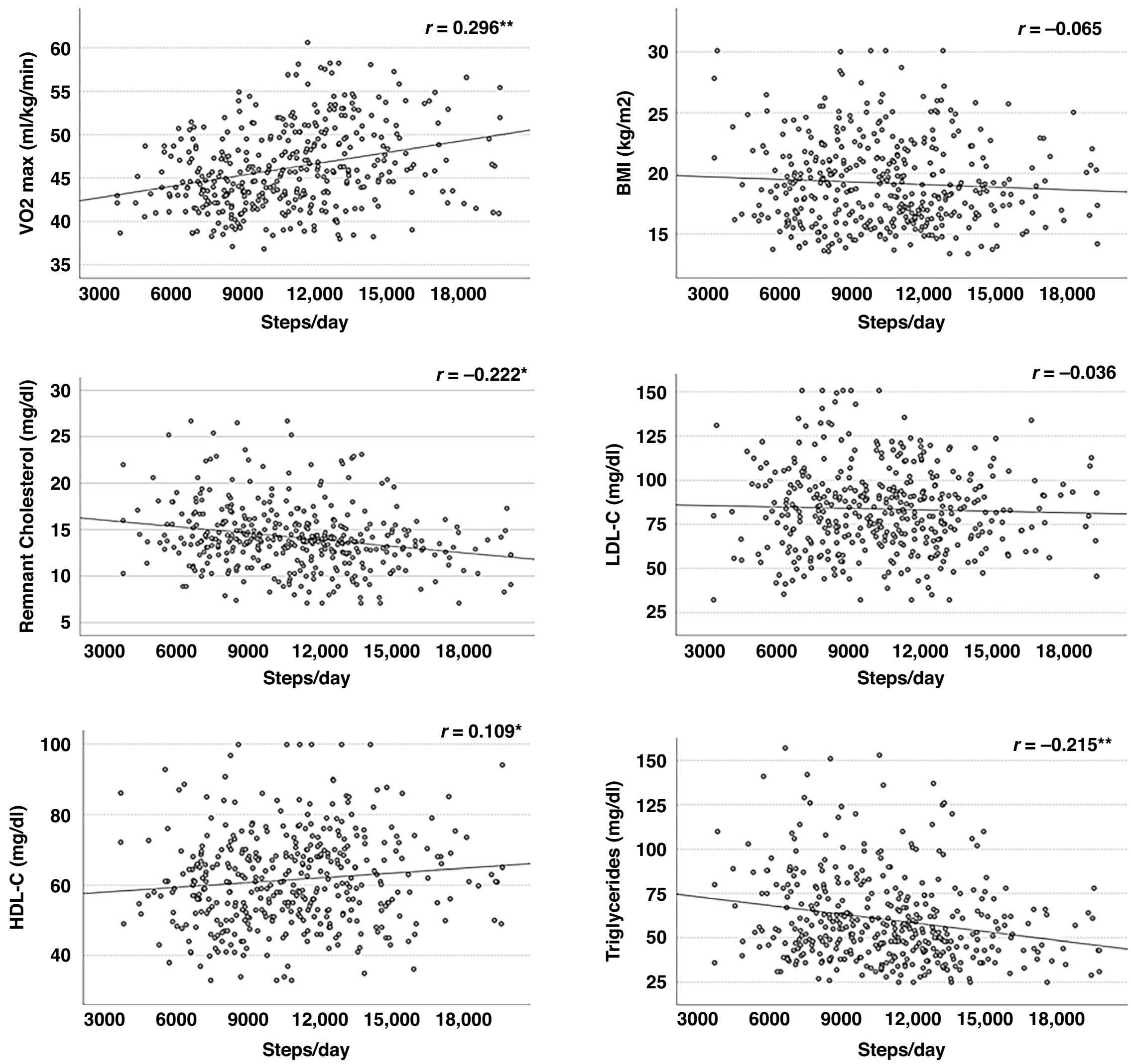

Fig. 2 Scatterplot showing bivariate correlation coefficients (*r*) between steps/day and CRF (VO2 max; maximal oxygen intake), body mass index (BMI), remnant cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, and total cholesterol. *p* value = $*p < 0.05$; $**p < 0.001$. The values in bold indicate statistical significance.

compensate for missing data based on an automatic chained method selected through comprehensive data analysis. This involved generating five replications, which were subsequently pooled together in the analysis.[45]

Analyses were performed using IBM SPSS Statistics version 29.0 (SPSS, Inc., Chicago, IL), with statistical significance defined as a two-tailed $p < 0.05$.

RESULTS

Table 1 shows the descriptive characteristics of the study participants (mean ± standard deviation). Of the 394 children included in the study, 185 were boys (53.0%), and the mean age was 9.96 ± 1.23 years. There were statistically significant differences between boys and girls in daily steps, CRF (VO2 max), remnant cholesterol, VLDL, and triglyceride levels.

The Pearson correlation coefficients between daily steps and CRF, BMI, remnant cholesterol, LDL-C, HDL-c, and triglyceride levels are shown in Fig. 2. Daily steps were positively correlated with CRF and HDL-c and inversely correlated with remnant cholesterol and triglycerides; however, the correlation coefficients were not significant for BMI or LDL-c. Furthermore, there was a strong and significant positive correlation between VLDL and remnant cholesterol, as well as an inverse correlation between VLDL and both daily steps and CRF (Table S3).

Figure 3a, b shows the mean differences in CRF and remnant cholesterol according to daily steps category using ANCOVA models. Children who accumulated >12,000 steps/day presented higher CRF levels than their peers with lower daily step counts, and those with 9000–12,000 steps/day had higher CRF levels than those with <9000 steps/day; moreover, there were no significant differences between those with >12,000 steps/day and those with 9000–12,000 steps/day when controlling for age, sex, and BMI. With regard to remnant cholesterol, walking >12,000 steps/day was associated with a lower daily step count; however, when controlling for age, sex, and BMI, the mean differences in remnant cholesterol were significant only when compared with taking <9000 steps/day. Finally, the significance of the difference disappeared after controlling for CRF. As shown in Fig. 3c, the differences in remnant cholesterol according to CRF category indicated that children in the high- and medium-CRF categories had lower levels of remnant cholesterol than their peers with lower CRF. After controlling for age, sex, BMI, and daily steps, remnant cholesterol was lower in children with better CRF than in those with lower CRF.

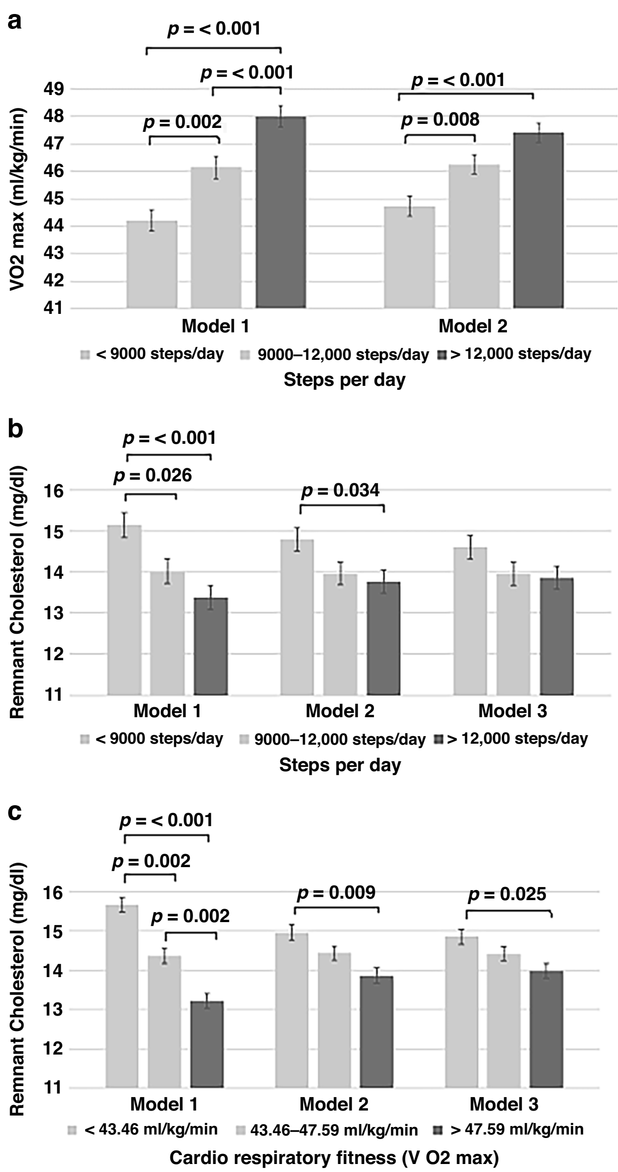

Fig. 3 Mean differences in cardiorespiratory fitness and remnant cholesterol using analysis of covariance models. a Cardiorespiratory fitness according to daily step categories. **b** Remnant cholesterol according to daily step categories. **c** Remnant cholesterol according to cardiorespiratory fitness categories. Model 0: crude data. Model 1: adjusted for age, sex, and body mass index. Model 2: Model 1 + cardiorespiratory fitness (B)/daily steps (**c**). The brackets indicate significant differences in the means ($p < 0.05$) between categories according to the Bonferroni multiple comparison post hoc test. Error bars represent the standard error.

Similar mean differences in CRF by daily steps category and mean differences in remnant cholesterol by CRF category showed similar results when multiple imputed data were used (Fig. S2A and S2C). When we examined the relationship between remnant cholesterol and daily steps, we observed that children with >12,000 steps/day had lower remnant cholesterol levels than their peers with fewer daily steps, and those children who walked between 9000 and 12,000 steps/day also had lower remnant cholesterol levels than those who walked <9000 steps/day (Fig. S2B).

The mediation analysis used to test whether the association between steps/day increment and remnant cholesterol was mediated by CRF is depicted in Fig. 4. In the first regression equation of the mediation model, daily step counts (every 1000 steps increment) showed a positive association with CRF levels (path a = 0.271, $p < 0.001$). According to the second regression equation, daily steps were inversely associated with remnant cholesterol levels (path c = −0.135; $p < 0.05$). In the final regression equation, the regression coefficient between daily steps and remnant cholesterol was reduced when the mediator (CRF) was included in the model (path c′ = −0.109; $p < 0.05$). Therefore, the mediation analysis indicates that each additional 1000 steps/day was associated with a greater CRF, which was positively associated with lower remnant cholesterol. Similar results were observed when multiple imputed data were used in the analyses (Fig. S3).

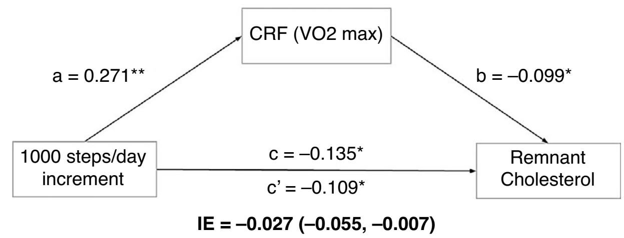

Fig. 4 Simple mediation model of the association between 1000 steps/day increment and remnant cholesterol using CRF (VO2 max; ml/kg/min) as a mediator and controlling for age, sex, and body mass index. The data are presented as unstandardised beta coefficients. *$p < 0.05$, **$p < 0.001$.

DISCUSSION

To our knowledge, this is the first study to examine the association between physical activity, measured objectively by daily steps, CRF and lipid profiles, including remnant cholesterol, a strong independent CVD risk factor, in children and to test whether the influence of physical activity on remnant cholesterol might be mediated by CRF levels. The main findings were that remnant cholesterol, but not LDL-c, was modifiable in those children who took >12,000 steps/day and had higher CRF levels, with the association between daily steps and remnant cholesterol being mediated by CRF (VO2 max).

Despite not being considered in most guidelines, remnant cholesterol has been recognised as an independent risk factor for CVD incidence, and its role in the development of atherosclerosis, even in individuals with normal LDL-c levels.[34] In a previous study, it was observed that children and adolescents with higher remnant cholesterol concentrations had elevated carotid intima-media thickness, which would represent a marker of early atherosclerotic damage.[46] This highlights its clinical relevance and places it as a potential therapeutic target in both adults and young people.[47]

The relationship between physical activity and CRF on the cardiometabolic profile and cardiovascular health is well established.[48,49] An increase in daily steps combined with an improvement in CRF leads to structural and functional adaptations in the cardiovascular system.[50] These adaptations include improved cardiac and pulmonary function, increased capillarization, and improved oxygen delivery to muscle, all of which contribute to more efficient lipid metabolism and improved cardiovascular health.[50,51] In this regard, our study showed enlightening results in children, indicating that a high volume of physical activity (>12,000 steps/day) is associated with higher levels of CRF (>47.59 ml/kg/min) and lower levels of remnant cholesterol. Likewise, it was noted that those children with higher levels of CRF were those with lower levels of remnant cholesterol. Therefore, physical activity may exert direct cardioprotective effects by reducing remnant cholesterol but also indirectly affect this lipid fraction by increasing the level of CRF. Additionally, considering the growing evidence supporting the crucial role of CRF in predicting survival and cardiovascular health, its assessment should be routine in clinical practice, as suggested by Bonikowske et al.[52]

Obesity seems to be the predominant cause of CVD risk in youth.[53] It has been demonstrated that increased adipose tissue is associated with an elevated synthesis and accumulation of remnant lipoproteins, which can be attributed to insulin resistance.[54,55] Pérez-Bay et al.[56] evidenced a bidirectional relationship between CRF and adiposity in children and adolescents, whereby higher CRF was negatively associated with obesity and higher fat mass was associated with lower levels of CRF. Certainly, physical activity or CRF affects obesity, but CRF may be an even more important predictor of CVD risk and long-term prognosis.[57] Additionally, school-based interventions that aim to enhance metabolic health and diminish adiposity during the early years of school may prevent progression of obesity and potentially cardiometabolic issues leading to CVD.[57,58] It is therefore plausible that such interventions, by reducing adiposity, may also contribute to lower remnant cholesterol levels.

These associations can be attributed to several metabolic mechanisms as follows. An increase in daily steps reflects increased physical activity, stimulates the body's energy expenditure and promotes fat oxidation;[59,60] together with CRF, this increase in daily steps improves insulin sensitivity[60,61] and reduces inflammation and oxidative stress.[62,63] All these factors lead to a reduction in triglyceride synthesis.[64–66] As a result, children with higher levels of daily physical activity tend to have lower triglyceride levels,[67] which is an important component of remnant cholesterol.[68]

Strengths and limitations

This study has several strengths, as it is the first study to investigate the relationship between physical activity, measured objectively by daily step count, CRF, and remnant cholesterol in a relatively large sample of schoolchildren. In addition, mediation analysis provides a better understanding of the underlying mechanisms relating physical activity, CRF, and cardiovascular health, although further studies are needed to consolidate this knowledge through prospective research.

However, some limitations need to be recognised. First, the cross-sectional design of the study prevents us from establishing causality in the observed associations. Longitudinal studies are needed to confirm the temporal relationships between daily steps, CRF, and remnant cholesterol. Second, the results of this study may not be generalizable to populations with different demographic characteristics or activity patterns because the study sample consisted exclusively of Spanish children in the 4th to 6th grades. Third, the children reported their daily steps on a weekly basis, which may have introduce inaccuracies in our physical activity data. Nevertheless, we made efforts to minimise bias and improve reliability by implementing a careful data collection process and providing clear instructions to the participants. Finally, given the exploratory nature of our mediation models, it is important to note that there are other covariates not considered that could have influenced the relationship between daily step count and remnant cholesterol.

CONCLUSION

In conclusion, this study highlights the associations between daily step count, CRF, and remnant cholesterol in schoolchildren. Increased daily physical activity is associated with higher CRF and lower remnant cholesterol levels, especially taking ≥9000 steps/day and >12,000 steps/day, respectively. In addition, CRF mediates the relationship between daily steps and remnant cholesterol. These results suggest that promoting physical activity in schoolchildren may be effective strategies for reducing remnant cholesterol and preventing CVD.

DATA AVAILABILITY

The datasets used and/or analysed in this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

Eva Rodríguez-Gutiérrez conceptualized and designed the study, drafted the initial manuscript, and critically reviewed and revised the manuscript. Vicente Martínez-Vizcaíno designed the the data collection instruments, collected data, and critically reviewed and revised the manuscript. Bruno Bizzozero-Peroni and Valentina Díaz-Goñi carried out the initial analyses, and critically reviewed and revised the manuscript. Irene Martínez-García, Irene Sequí-Domínguez, and Sergio Núñez de Arenas-Arroyo collected data, and critically reviewed and revised the manuscript. Mairena Sánchez-López and Carlos Pascual-Morena designed the study, collected data, and critically reviewed and revised the manuscript. Ana Torres-Costoso coordinated and supervised data collection, and critically reviewed and revised the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Clinical Research Ethics Committee of the Hospital Virgen de la Luz in Cuenca (REG: 2019/PI1519). After the Board of Governors of each school approved the study, a letter was sent to the parents of all 4th, 5th and 6th graders inviting them to a meeting. At this meeting, we explained the objectives of the study and asked for written approval for their children's participation. All procedures performed in this study were in accordance with the Declaration of Helsinki and its later amendments or comparable ethical standards for experiments involving humans.[35]

ADDITIONAL INFORMATION

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