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# Reallocating time spent in physical activity intensities: longitudinal associations with physical fitness (DADOS Study) 

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#### Abstract

Objectives: Firstly, to investigate the longitudinal associations between accelerometer-derived physical activity (PA) intensities and physical fitness (PF) at 24-month follow-up in adolescents. Secondly, to examine how substituting time spent in low or moderate PA intensities with vigorous PA at baseline was related to PF at 24-month follow-up.

Design: Longitudinal observational study Method: The DADOS (Deporte, ADOlescencia y Salud) study is a 3-year longitudinal research project carried out between years 2015-2017. The analyses included 189 adolescents ( 91 girls) aged 13.9 $\pm 0.3$ years at baseline. PA was assessed by a wrist-worn GENEActiv triaxial accelerometer and expressed as minutes/day of light, moderate and vigorous PA. Cardiorespiratory, musculoskeletal and motor fitness were assessed by field tests and a global fitness $z$-score was calculated as the mean of the z -scores values of each fitness test. Association between PA intensities and PF were determined using linear regression. Isotemporal analyses estimating the association of reallocating PA intensities with PF were performed. Results: Baseline vigorous PA was positively associated with cardiorespiratory fitness and global fitness score at follow-up in boys ( $\beta=0.234 ; \mathrm{p}=0.002, \beta=0.340 ; \mathrm{p}<0.001$ ) and girls ( $\beta=0.184 ; \mathrm{p}=0.043$, $\beta=0.213 ; p=0.004$ ). In boys, baseline vigorous PA was also positively associated with musculoskeletal and motor fitness ( $\beta=0.139 ; p=0.035, \beta=0.195 ; p=0.021$ ). The substitution of $10 \mathrm{~min} / \mathrm{day}$ of light PA or


moderate PA with $10 \mathrm{~min} /$ day of vigorous PA at baseline was positively associated with all PF components and global fitness score in boys ( $\mathrm{p}<0.001$ ), and with global fitness score girls ( $\mathrm{p}<0.05$ ).

Conclusion: These findings highlight the need of promoting vigorous PA due to its specific influence on adolescents' PF.

Key words: vigorous intensity; fitness; health; behaviour; adolescence.

## Introduction

Physical fitness (PF) is considered a powerful health marker during youth due to its role in disease prevention, which main components are cardiorespiratory, musculoskeletal and motor capacities ${ }^{1}$. The evidence of health-related benefits in children and adolescents is particularly strong for cardiorespiratory fitness ${ }^{2}$; however, emerging research supports also the preventive role of musculoskeletal fitness ${ }^{3,4}$ as well as motor fitness ${ }^{5}$. Although PF is highly determined by biological factors (e.g. genetic heritability, sex, age, pubertal status) ${ }^{6,7}$, physical activity (PA) is suggested to be the most modifiable determinant ${ }^{8}$.

PA is commonly considered to be closely interconnected with PF, but this assumption is not so obvious in adolescents, probably due to the complexity of assessing the whole range of components of this behaviour ${ }^{9}$. Advances in the assessment of PA by accelerometer has improved the accuracy and has allowed to quantify the amount of time spent in specific free-living PA intensities ${ }^{10}$. Previous crosssectional studies in adolescents reported that PF may be related to the level of accelerometer-measured PA intensities, suggesting a stronger association of vigorous PA than moderate and light PA intensities with cardiorespiratory fitness ${ }^{11-13}$, musculoskeletal and motor fitness ${ }^{14,15}$. Nevertheless, there is a lack of data regarding the longitudinal association of accelerometer-derived free-living PA intensities with PF components in adolescents. In fact, only two studies investigated cardiorespiratory fitness ${ }^{16,17}$, whereas musculoskeletal and motor fitness have not been investigated.

According to the aforementioned knowledge, there is a need for further longitudinal studies assessing free-living PA intensities by accelerometer to clarify if they could predict PF over time in adolescents. Moreover, there is not previous research that investigated the effects elicited by substituting time in a given PA intensity with an equal amount time in another intensity on PF, which currently can be analysed by isotemporal substitution models ${ }^{18}$. This statistical method is based on the finiteness of time in any given 24h period. Thus, participating in a specific behaviour necessarily implies reducing time in another of equal duration. Altogether, filling these gaps of knowledge would help to better stablish preventive strategies and intervention programs targeting health in adolescents.

The first aim of this study was to investigate the longitudinal associations between accelerometermeasured PA intensities and PF components (i.e. cardiorespiratory, muscular and motor fitness) at 24-
month follow-up in adolescents. The second aim was to examine how substituting time spent in light or moderate PA intensities with vigorous PA at baseline was related to PF at 24-month follow-up using isotemporal substitution regression models.

## Methods

The present study is part of the DADOS (Deporte, ADOlescencia y Salud) study, a 3-year longitudinal research project carried out between February and May of 2015 and 2017 which aimed to investigate the influence of PA on health and development in adolescents. All participants were volunteers recruited from secondary schools and sports clubs of Castellon (Spain), and met the general DADOS inclusion criteria: to be enrolled in the 2 nd grade of secondary school, and free of any chronic disease. Participants who reported medical contraindication for maximum physical effort or failed a previous academic year were excluded from the study. A total of 189 adolescents ( 91 girls) aged $13.9 \pm 0.3$ years at baseline with valid data at baseline and follow-up for PF, PA, body mass index (BMI) and pubertal status were included in the analyses. Adolescents and their parents or guardians were informed of the nature and characteristics of the study, and all provided a written informed consent. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Fortaleza 2013), and the study protocol was approved by the Research Ethics Committee of the University Jaume I (Spain). PA intensities were measured using the GENEActiv accelerometer (Activinsights Ltd, Kimbolton, UK), a waterproof device which contains a triaxial microelectromechanical accelerometer that records both motion-related and gravitational acceleration and has a linear and equal sensitivity along the three axes. Participants wore the accelerometer on their non-dominant wrist. GENEActiv accelerometer offers a body temperature sensor to help improve the confirmation of wear and non-wear time; yet in order to check possible inconsistencies in the accelerometer data, participants kept a physical activity log of the number of times and duration that they removed it. A high level of agreement was observed between the accelerometer and the participants' activity $\log$ data. Accelerometer-derived data from all participants comprised at least four days, including weekend and weekdays, with 24 h valid data. GENEActiv accelerometer has been found to be a reliable (Coefficient of Variation intra-instrument = $1.4 \%$, Coefficient of Variation inter-instrument $=2.1 \%$ ) ${ }^{19}$ and valid measure of PA in young people ( r
$=0.925, \mathrm{P}=0.001)^{20}$. Devices were programmed with a sampling frequency of 100 Hz , and data were stored in gravity ( g ) units ( $1 \mathrm{~g}=9.81 \mathrm{~m} /$ second $^{2}$ ). The raw acceleration output was converted to 1 second epochs using the GENEActiv Post-Processing PC Software (version 2.2, GENEActiv). By combining all registered days for each participant and according to Phillips et al. ${ }^{20}$, PA was expressed as the average ( $\mathrm{min} /$ day $)$ of light, moderate and vigorous PA.

Cardiorespiratory fitness was assessed using the 20-m Shuttle Run Test. CRF was assessed using the $20-\mathrm{m}$ Shuttle Run Test as described by Léger et al. ${ }^{21}$. Each participant ran straight between 2 lines 20 m apart at a pace established by recorded audio signals. The initial speed was $8.5 \mathrm{~km} / \mathrm{h}$ and it was increased $0.5 \mathrm{~km} / \mathrm{h}$ each minute. The test was completed when participants could not reach the end lines at the pace of the audio signals for 2 consecutive times or when they stopped because of fatigue. The number of completed laps was used in the analyses.

Musculoskeletal fitness was assessed trough the standing broad jump test ${ }^{22}$. From a starting position immediately behind a line, standing with feet approximately shoulder's width apart, the adolescent jumped as far as possible with feet together. The test was performed twice and the longest distance achieved (centimetres) was used in the analyses.

Motor fitness was assessed using the $4 \times 10-\mathrm{m}$ shuttle-run test of speed of movement, agility, and coordination ${ }^{22}$. Subjects ran back and forth as fast as possible between two parallel lines 10 m apart. Every time the adolescent crossed any of the lines, he or she picked up (the first time) or exchanged (second and third time) a sponge, which was previously placed behind the lines. The test was performed twice and the fastest time (seconds) was used in the analyses. Because motor fitness is inversely related to high PF (longer time indicates poorer performance), the variable expressed in seconds was inverted by multiplying by -1 , so that a higher score indicates better performance.

The individual score of each fitness test was transformed into sex-specific standardized values (zscores). A global fitness score was calculated as the mean of the z -scores values of cardiorespiratory, musculoskeletal and motor fitness. Higher z-score values in global fitness indicate better performance.

Pubertal status was self-reported according to the 5 stages described by Tanner and Whitehouse ${ }^{23}$. It is based on external primary and secondary sex characteristics, which are described by the participants using standard pictures according to Tanner instructions.

BMI was calculated as weight/height square $\left(\mathrm{kg} / \mathrm{m}^{2}\right)$. Body weight was measured to the nearest 0.1 kg using an electronic scale (SECA 861, Hamburg, Germany). Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (SECA 213, Hamburg, Germany). Measures were assessed in duplicate and average measures were used for the analyses.

Descriptive sample characteristics were summarized by sex and were presented as mean $\pm$ standard deviation (SD). Comparisons between sexes at each time point were performed by independent $t$ test for continuous variables and chi- squared test for nominal variables. Similarly, differences between descriptive data at baseline and at 24 -month follow-up were assessed by paired $t$ test or chi- squared test.

Linear regression analyses were conducted to assess the association between time spent in specific PA intensities at baseline (min/day) with each PF indicator at 24-month follow-up. Each PA intensity (light, moderate or vigorous) at baseline was entered as the independent variable and each PF indicator was entered as dependent variable in separate models. Due to the influence of age, pubertal status and BMI on PF and PA levels, all models were controlled for the baseline scores of these factors ${ }^{6}$.

In addition, we performed isotemporal substitution models to examine the effect of replacing one type of PA intensity for another PA intensity at baseline on PF at 24-month follow-up, assuming that the time spend in non-PA tasks is constant in a 24 h -period ${ }^{18}$. First, all variables were scaled to 10 minutes to aid in interpretability, and then a "total time" score was calculated to represent the average daily time spent in the different PA intensities (i.e., total time $=$ light + moderate + vigorous PA). Next, total time, all PA intensities, and covariates (i.e., age, pubertal status and BMI) were entered into a regression model simultaneously with the exception of the PA intensity of interest. An example model for light PA can be expressed as follows:

Cardiorespiratory fitness $=\left(\mathrm{B}_{1}\right)$ vigorous $\mathrm{PA}+\left(\mathrm{B}_{2}\right)$ moderate $\mathrm{PA}+\left(\mathrm{B}_{3}\right)$ total time $+\left(\mathrm{B}_{4}\right)$ covariates

Where $B_{1}$ to $B_{4}$ represent the coefficients of respective activities or covariates included in the model. Because in this example light PA would be omitted from the model, the remaining coefficients represent the consequence of engaging in 10 minutes of the respective PA instead of engaging in 10 minutes of light PA while holding the other activities constant (e.g., B 1 represents the change in cardiorespiratory fitness if 10 minutes of light PA are substituted with 10 minutes of vigorous PA while holding other intensities and covariates constant). Therefore, results are interpreted as unit change in fitness score per 10 -minute change in the specific PA intensity. The $\mathrm{B}_{2}$ coefficient would provide the same information in relation to moderate PA. Models substituting the three PA intensities (light, moderate, vigorous) were performed for all PF indicators in separate models.

Due to the sex differences in $\mathrm{PA}^{24}$ and PF levels ${ }^{6}$ reported in the literature, as we all those found in our sample, the analyses were performed separately by sex. Multicollinearity was checked using the variance inflation factor, which values were less than 2 in all analysis, indicating that multicollinearity was low. All the analyses were performed using the IBM SPSS Statistics for Windows version 22.0 (Armonk, NY: IBM Corp). The level of significance was set at $\mathrm{p}<0.05$.

## Results

The characteristics of the adolescents at baseline and at 24-month follow-up segmented by sex are shown in Table 1. Baseline BMI was greater in girls than boys ( $\mathrm{p}=0.04$ ), and both sexes increased BMI at follow-up ( $\mathrm{p}<0.001$ ). Boys performed higher levels of moderate and vigorous PA than girls at baseline and at follow-up (all p<0.01). Time spent in specific PA intensities decreased significantly in both boys and girls (all $\mathrm{p}<0.01$ ). Boys showed higher levels of PF than girls at baseline and at follow-up ( $\mathrm{p}<0.001$ ). All PF components significantly increased in boys (all $\mathrm{p}<0.001$ ), whereas girls only showed increased levels of motor fitness ( $\mathrm{p}<0.001$ ).

The associations between PA at baseline and PF at 24-month follow-up are shown in Table 2. Baseline vigorous PA was positively associated with cardiorespiratory fitness and global fitness score at followup in boys and girls (all $\mathrm{p}<0.05$ ). Moreover, baseline vigorous PA was positively associated with musculoskeletal ( $\mathrm{p}=0.035$ ) and motor fitness ( $\mathrm{p}=0.021$ ) in boys.

Isotemporal substitution analyses are shown in Table 3. In boys, the substitution of 10 min /day of light or moderate PA with $10 \mathrm{~min} /$ day of vigorous PA at baseline was positively associated with cardiorespiratory, musculoskeletal and motor fitness, as well as with global fitness score (all $\mathrm{p}<0.05$ ). In girls, the substitution of $10 \mathrm{~min} /$ day of light PA with $10 \mathrm{~min} /$ day of vigorous PA at baseline was almost significantly associated with CRF ( $\mathrm{p}=0.059$ ). In addition, the substitution of 10 min /day of light or moderate PA with $10 \mathrm{~min} /$ day of vigorous PA at baseline was positively associated with global fitness score (all $\mathrm{p}<0.05$ ). Substituting light PA with moderate PA was not associated with any of the PF indicators ( $\mathrm{p}>0.05$, data not shown).

## Discussion

The main finding of this study was the intensity-specific association between free-living PA and PF in adolescents. Indeed, our results showed that the level of vigorous PA at baseline was positively associated with PF 24-months later, revealing sex-specific differences. Results of the isotemporal analyses indicated that only substitution of light or moderate PA with vigorous PA was positively associated with PF at 24-month follow-up. These findings contribute to the scarce current literature about the longitudinal association between accelerometer-measured free-living PA and PF in adolescents, and add knowledge about the influence of replacing time spent in specific PA intensities on PF.

With regard to the cardiorespiratory fitness, our results agree with the few previous longitudinal studies investigating the association between accelerometer-derived PA intensities and this fitness capacity, which also showed unique associations with vigorous PA in adolescents, but without examining sex differences ${ }^{16,17}$. Our findings could also be supported by interventional studies aimed to improve cardiorespiratory fitness, which have shown that cardiorespiratory fitness is mostly improved by PA at the higher end of the intensity spectrum in children and adolescents ${ }^{25}$. The isotemporal analyses support this previous evidence by the fact that only replacing light and moderate intensities with vigorous PA was positively associated with cardiorespiratory fitness in our group of adolescents.

Concerning to musculoskeletal and motor fitness, we also found that greater time spent in vigorous PA intensity at baseline, as well as substituting light and moderate intensities with vigorous PA, was significantly associated with these PF components in boys but not in girls. Motor fitness was the only PF component that significantly improved from baseline to follow-up in girls; however, it was not associated with PA intensity. These results could be due to the fact that changes in this fitness capacity may not be linearly related to the PA performed due to different patterns of change within the group of girls. To the best of our knowledge, no previous longitudinal study has explored the association of accelerometer-derived PA intensities with musculoskeletal and motor fitness in adolescents. Yet, our findings are supported by some of the limited previous cross-sectional studies investigating the association with musculoskeletal fitness ${ }^{14,15}$, which also reported unique associations with vigorous PA. The sex differences observed in our sample could be explained by the lower time spent in daily vigorous PA in girls compared to boys, which may not be sufficient to induce musculoskeletal fitness adaptations ${ }^{26}$.

With respect to the global fitness score, in boys, baseline vigorous PA was positively associated with global fitness score at follow-up, with a higher standardized regression score than PF components individually. In girls, isotemporal substitution analyses showed a positive association with global fitness, despite the fact that PF components independently showed not significant associations. These results could be partially explained by the cumulative influence of each PF component on the global fitness score.

All in all, the unique associations found in the present study between vigorous PA and PF may be related to the specific physiological stimuli exerted by this intensity. Vigorous PA requires a higher degree of work performed per unit of time compared to light or moderate intensities. Therefore, from a physiological point of view, it may require specific neuromuscular activation patterns such as greater motor-unit recruitment of type II fibres, discharge rate and/or synchronization ${ }^{27,28}$, which induce specific adaptations like greater rate of force development ${ }^{28,29}$. In addition, regular vigorous PA may induce particular metabolic stimulus influencing muscular and cardiometabolic systems adaptations like mitochondrial biogenesis ${ }^{30}$, translating into greater oxidative capacity and cardiorespiratory capacity.

Our results are of relevance since providing insight in the effects of PA intensities may help authorities, educators and clinicians in designing the optimal guidance on how to perform health-enhancing PA. From a practical point of view, our findings indicate that it is relevant to assess the PA behaviour considering the different intensities separately, since reporting them combined in moderate and vigorous or total PA may hinder important specific effects of this behaviour. Moreover, our results suggest that vigorous PA should be promoted and encouraged in any of the context where adolescents can engage (i.e. organized sport, recreational active play or school-based PA) ${ }^{31,32}$, since it may have important public health impact given the benefits of improved PF on health. Adolescents should not be treated as a whole homogenous group in the attempt to promote PF due to the sex-differences in the vigorous PA impact.

An important strength of our study is the longitudinal design with a homogenous sample in terms of age and sex, as well as the inclusion of isotemporal models to assess the relationship between PA intensities and PF components. In addition, we assessed PA by accelerometer, which has several advantages over questionnaires and self-report methods, although some controversies have also been reported ${ }^{10,33}$. Valid and reliable tests were used to assess $\mathrm{PF}^{22}$, which is feasible and time-efficient in observational studies; however, we acknowledge that more accurate data could be obtained in laboratory settings. We controlled the analyses for several potential cofounders, but other unmeasured confounders such as genetic, environmental or social factors could have influenced our results. Finally, it is important to mention that despite significant associations were found in this study, the effect sizes were weak (the $\beta$ standardized regression coefficient ranged between 0.139 and 0.368 ).

In conclusion, our results support that free-living vigorous PA is associated with better PF over time in adolescents, with sex-specific relationships for each fitness capacity. These findings highlight the need of targeting specifically vigorous PA within the existing general PA recommendations for adolescents.

## Practical Implications:

- Our findings highlight a positive relationship between free-living vigorous physical activity at baseline and physical fitness indicators 24-months later.
- Replacing light or moderate physical activity with vigorous physical activity was positively associated with physical fitness indicators at 24-month follow-up.
- Assessment of the health-related effects of physical activity behavior should be performed taking into consideration the different intensities separately.
- Vigorous physical activity should be specifically encouraged in future health-related promotional strategies for adolescents due to its unique influence on physical fitness.
- Adolescents should not be treated as a whole homogenous group in the attempt to promote physical fitness due to the sex-differences in the vigorous physical activity impact.

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0.

Table 1. Characteristics of participants at baseline and at 24-month follow-up (boys $n=98$; girls $n=91$ ).

|  | Baseline |  | Follow-up |  | p values * |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Boys | Girls | Boys | Girls | Boys | Girls |
| Age (years) | 13.9 (0.3) | 13.9 (0.3) | 15.8 (0.3) | 15.8 (0.3) | <0.001 | <0.001 |
| Weight (kg) | 54.0 (9.5) | 53.4 (9.0) | 64.0 (8.7)** | 58.4 (9.1) | $<0.001$ | $<0.001$ |
| Height (cm) | 164.7 (8.5)** | 160.7 (6.8) | 172.7 (6.6)** | 162.7 (6.6) | <0.001 | <0.001 |
| BMI ( $\mathrm{kg} / \mathrm{m}^{2}$ ) | 19.8 (2.5)** | 20.6 (2.9) | 21.4 (2.6) | 22.1 (3.2) | <0.001 | $<0.001$ |
| Tanner stage (I-V) (\%) | 0/8/33/45/14 | 0/7/34/55/4 | 0/0/4/39/57** | 0/0/15/70/15 | <0.001 | $<0.001$ |
| Physical activity levels (min/day) |  |  |  |  |  |  |
| Light | 176.4 (68.9) | 170.1 (24.6) | 154.9 (30.4) | 157.6 (27.9) | <0.001 | $<0.001$ |
| Moderate | 81.5 (26.4)** | 69.1 (18.0) | 59.1 (33.1)** | 43.1 (33.6) | <0.001 | $<0.001$ |
| Vigorous | 15.2 (7.8)** | 9.4 (8.0) | 9.2 (7.6)** | 4.3 (6.5) | <0.001 | $<0.001$ |
| Physical Fitness |  |  |  |  |  |  |
| Cardiorespiratory fitness (laps) | 78.7 (20.6)** | 52.3 (21.0) | 87.5 (19.4)** | 51.5 (20.5) | $<0.001$ | 0.520 |
| Musculoskeletal fitness (cm) | 181.5 (24.6)** | 162.1 (25.0) | 206.5 (25.9)** | 162.3 (30.2) | $<0.001$ | 0.913 |
| Motor fitness (s) | 12.0 (0.7)** | 13.0 (0.9) | 11.0 (0.7)** | 12.4 (0.8) | <0.001 | $<0.001$ |
| Global fitness score ${ }^{\dagger}$ | 0.02 (0.87) | 0.07 (0.92) | -0.01 (0.40) | -0.02 (0.45) | 0.739 | 0.129 |

Data are shown as mean (standard deviation). * Differences between baseline and follow-up tested by paired test or chi- squared test. ${ }^{* *}$ Differences between sexes tested at the specific time point by independent $t$ test or chi-squared test ( $\mathrm{p}<0.05$ ).
${ }^{\dagger}$ The global fitness score was calculated as the mean of the z -scores values of cardiorespiratory, musculoskeletal and motor fitness.

Table 2. Associations between physical activity levels at baseline and physical fitness at 24 -month follow-up by sex.

|  | Cardiorespiratory fitness |  |  | Musculoskeletal fitness |  |  | Motor fitness ${ }^{\dagger}$ |  |  | Global fitness score * |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\beta$ | 95\% CI | p | $\beta$ | 95\% CI | p | $\beta$ | 95\% CI | p | $\beta$ | 95\% CI | p |
| Boys ( $n=98$ ) |  |  |  |  |  |  |  |  |  |  |  |  |
| Light PA | 0.004 | -0.038; 0.040 | 0.954 | -0.016 | -0.055; 0.042 | 0.803 | 0.049 | -0.001; 0.002 | 0.561 | 0.017 | -0.001; 0.001 | 0.826 |
| Moderate PA | 0.092 | -0.035; 0.170 | 0.192 | -0.034 | -0.163; 0.097 | 0.616 | 0.096 | -0.002; 0.007 | 0.260 | 0.074 | -0.001; 0.004 | 0.349 |
| Vigorous PA | 0.234 | 0.232; 0.949 | 0.002 | 0.139 | 0.032; 0.894 | 0.035 | 0.195 | 0.003; 0.031 | 0.021 | 0.340 | 0.010; 0.025 | <0.001 |
| Girls ( $n=91$ ) |  |  |  |  |  |  |  |  |  |  |  |  |
| Light PA | -0.008 | -0.107; 0.094 | 0.894 | -0.072 | -0.304; 0.127 | 0.416 | -0.021 | -0.006; 0.005 | 0.792 | 0.012 | -0.002; 0.002 | 0.842 |
| Moderate PA | 0.042 | -0.096; 0.192 | 0.666 | -0.048 | -0.381; 0.219 | 0.593 | 0.003 | -0.008; 0.008 | 0.969 | 0.056 | -0.002; 0.005 | 0.372 |
| Vigorous PA | 0.184 | 0.016; 0.933 | 0.043 | -0.164 | -1.359; 0.120 | 0.100 | 0.277 | -0.008; 0.028 | 0.246 | 0.213 | 0.004; 0.020 | 0.004 |

Data are presented as standardized regression coefficient ( $\beta$ ) and $95 \%$ CI. Analyses were adjusted for age, pubertal status, body mass index and the corresponding physical fitness value at baseline. Statistically significant values ( $\mathrm{p}<0.05$ ) are in bold.
PA: physical activity; CI: confidence intervals.
${ }^{\dagger}$ The original score expressed in seconds was multiplied by -1 , so that a higher score indicates better performance.
${ }^{\ddagger}$ The global fitness score was calculated as the mean of the z -scores values of cardiorespiratory, musculoskeletal and motor fitness.

Table 3. Isotemporal substitution models examining the association between replacing 10-min of light or moderate physical activity with vigorous physical activity at baseline and physical fitness at 24-months follow-up.

|  | Boys |  |  |  | Girls |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | B | $\beta$ | 95\% CI | p value | B | $\beta$ | 95\% CI | p value |
| Cardiorespiratory fitness |  |  |  |  |  |  |  |  |
| Replacing light PA | 5.492 | 0.217 | 1.566; 9.418 | 0.007 | 4.732 | 0.184 | -0.182; 9.644 | 0.059 |
| Replacing moderate PA | 5.439 | 0.216 | 1.034; 9.844 | 0.016 | 4.730 | 0.184 | -1.388; 10.848 | 0.128 |
| Musculoskeletal fitness |  |  |  |  |  |  |  |  |
| Replacing light PA | 5.583 | 0.174 | 1.142; 10.425 | 0.015 | -6.139 | -0.162 | -14.504; 2.279 | 0.148 |
| Replacing moderate PA | 6.753 | 0.203 | 1.424; 12.083 | 0.014 | -8.790 | -0.232 | -19.708; 2.129 | 0.113 |
| Motor fitness ${ }^{\dagger}$ |  |  |  |  |  |  |  |  |
| Replacing light PA | 0.231 | 0.266 | 0.079; 0.382 | 0.003 | 0.117 | 0.112 | -0.090; 0.324 | 0.264 |
| Replacing moderate PA | 0.279 | 0.322 | 0.105; 0.453 | 0.002 | 0.175 | 0.168 | -0.094; 0.444 | 0.200 |
| Global fitness score ${ }^{\text { }}$ |  |  |  |  |  |  |  |  |
| Replacing light PA | 0.187 | 0.357 | 0.106; 0.268 | <0.001 | 0.122 | 0.217 | 0.034; 0.211 | 0.007 |
| Replacing moderate PA | 0.193 | 0.368 | 0.101; 0.285 | <0.001 | 0.132 | 0.234 | 0.019; 0.244 | 0.022 |

PA: physical activity. The unstandardized regression coefficient (B) with its $95 \%$ confidence interval (CI), the standardized regression coefficient ( $\beta$ ) and the $p$ value are given for each association.
Isotemporal Substitution Models were adjusted for age, pubertal status, body mass index and the corresponding physical fitness value at baseline.
${ }^{\dagger}$ The original score expressed in seconds was multiplied by -1 , so that a higher score indicates better performance.

* The global fitness score was calculated as the mean of the z-scores values of cardiorespiratory, musculoskeletal and motor fitness.

