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ORIGINAL PAPER

Ace I/D polymorphism is associated with habitual physical activity in pubertal boys

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Abstract We investigated the association between the angiotensin I-converting enzyme (ACE) gene insertion (I)/deletion (D) polymorphism and physical activity levels in boys at early pubertal stage (calendar age 12.04 ± 0.77 years). Body composition by DXA, pubertal stage and cardiovascular fitness on cycle ergometer were measured in addition to 7-day accelerometry. DNA was separated from the whole blood. Sedentary behaviour level was significantly lower in DD subjects compared to I allele carriers. A significant main effect of the D allele was found on total physical activity ($F_{1,256} = 5{,}453$; $p = 0.020$; $\eta^2 = 0.021$] and on light physical activity ($F_{1,256} = 4.74$; $p = 0.030$; $\eta^2 = 0.018$). Adding screen time as a covariate did not change ACE I/D polymorphism effect on total physical activity levels ($F_{2,256} = 3{,}326$; $p = 0.041$; $\eta^2 = 0.025$). Carriers of the D allele had significantly higher light physical activity ($F_{1,256} = 4{,}710$; $p = 0.031$; $\eta^2 = 0.20$), with screen time as covariate. In conclusion, ACE gene has a significant effect on sedentary, light and total physical activity levels in healthy 12-year-old boys.

Keywords Physical activity · Sedentary behaviour · ACE gene · Pubertal boys

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Introduction

Physical activity is considered as a major risk factor for complex metabolic diseases such as diabetes, hypertension and obesity. Some authors have suggested that children are nowadays less active and spend more time in sedentary activities [1]. However, not all children choose the sitting lifestyle, and it is likely that the behaviour of physical activity may have genetic influence [2]. Despite the fact that twin studies indicate heritability to account for up to 68 % of physical activity [3], it has also been shown that genetic factors are modest determinants of being physically active, and the environment is more important determinant [4].

Human studies have pointed a number of candidate genes, which may interact with physical activity and environmental parameters. Angiotensin-converting enzyme (ACE) catalyses the conversion of angiotensin I to angiotensin II and the breakdown of the vasodilator bradykinin to kinin degradation products which have several effects on cardiovascular system [5]. The functional ACE insertion (I) and deletion (D) polymorphism on the presence of a 287-bp *Alu* repeat sequence within intron 16 is one of the most common genetic variants that has been shown to be related to ACE levels in blood [6] and increased cardiovascular risks [7]. ACE has also been found to be related to several cardiovascular fitness components (for review, see [8]) that could make the ACE gene a good candidate for an influence on physical activity. Subjects with the II genotype have higher levels of kinins that increase local blood flow, oxygen and substrate delivery to muscles [9], and glucose uptake in muscles [10] that could result in higher physical performance and energy availability for movement and activity. Additionally, the renin–angiotensin system is also present in the central nervous system and

may exert a central influence on predisposition to being physically active [11].

There are only very limited data on specific gene interactions with habitual physical activity levels. Most of the published studies on candidate gene associations to exercise behaviour or related physical activity phenotypes have used questionnaires in order to quantify different levels of physical activity [12–14]. Studies on interactions of the ACE gene and physical activity have been conflicting. For example, Winnicki et al. [14] investigated the ACE I/D polymorphism association with physical activity levels in a heterogeneous group of subjects with mild hypertension. They indicated that ACE I/D polymorphism may be a specific genetic factor associated with physical activity levels. In contrast, no relationship between the ACE gene and moderate to vigorous physical activity (MVPA) were found in 3- to 12-year-old children [15]. Other studies with other different genes have found an association with genotype and accelerometry-based physical activity [13, 16, 17]. However, most studies in the field have considered physical activity rather as a covariate for the gene effects on other phenotypes, rather than the dependent variable itself.

Genetic influence on physical activity is possibly more easy to detect in children compared to adults [4], probably because the levels of physical activity during childhood has more variation compared to adults, as the physical activity of an adult is highly directed by work and non-genetic environmental influences. The development of accelerometry enables the measurement of different levels of physical activity more objectively. It also allows the monitoring of free-living subjects over a number of days, and the study of health effects of all physical activity intensity levels [15, 18]. The aim of the present study was to investigate associations between ACE I/D polymorphism and different physical activity levels in healthy boys at early pubertal stage, and we hypothesized that I allele would be related to higher physical activity.

Methods

Subjects

In total, 314 fifth and sixth grade boys were recruited from schools in the Tartu county, Estonia. The sampling unit for the study was a school class. The overall participation rate was 74 %. Valid and complete data were unavailable for 49 (16 %) participants; thus, the final sample available for analysis consisted of 261 boys (calendar age 12.04 ± 0.77 years). All children and parents were informed of the purpose and content of the study. Before participation, parents signed a written consent and children gave their verbal assent. This study was approved by the Medical Ethics Committee of the University of Tartu and was according to the Declaration of Helsinki.

Anthropometry

Body height (cm) was measured in a standing position to the nearest 0.1 cm using a Martin metal anthropometer according to the standard technique. Body mass (kg) was measured with minimal clothing using medical scales (A&D Instruments, UK) to the nearest 0.05 kg, and body mass index (BMI; kg/m^2) was calculated. Body fat percent was measured using a DPX-IQ densitometer (Lunar, Madison, WI, USA) with the subject lying in light clothing and arms to the sides. The apparatus was calibrated according to the suggestions of the manufacturer and was run in medium scan mode. The biological age of the participants was assessed according to the self-assessment using an illustrated questionnaire of the pubertal stage according to the Tanner classification method [19]. Evaluation of pubic hair was used.

Assessment of cardiovascular fitness (CVF)

CVF was determined on an electrically braked bicycle ergometer (Corival; Lode, Netherlands) using a stepwise incremental exercise test until volitional exhaustion. The initial work rate was 60 W with the increments of 25 W after every third minute. Pedal frequency was set at 60–75 rpm. Subjects were verbally encouraged to produce maximal effort. The criteria for the maximal effort were: (1) the inability to sustain the required work intensity, and (2) heart rate >95 % of their age-predicted maximum (220-age). Maximal aerobic power (Pa_{max}, W) was calculated according to the following formula [20]:

$$Pa_{max} = W1 + (W2 \times t/180),$$

where $W1$ is workload (W) at each fully completed stage, $W2$ is workload increment at the final incomplete stage, and t is the duration (s) of the final incomplete stage. Maximal aerobic power output per kilogram of body mass ($Pa_{max/kg}$, W/kg) was calculated.

Measurement of physical activity

Physical activity was objectively measured by accelerometry (GT1M ActiGraph; Monrovia, CA, USA). The accelerometer used was a compact, small (3.8 × 3.7 × 1.8 cm), lightweight (27 g), and uniaxial monitor designed to detect vertical accelerations raging in magnitude from 0.05 to 2.00 Gs with a frequency response of 0.25–2.50 Hz. The accelerometer has been previously validated in laboratory and free-living conditions in young people [21].

Each participant was asked to carry the monitor on the hip for seven consecutive days. The accelerometer was programmed to record the activity counts in 60-s epochs. For the analysis of accelerometer data, all sequences of 10 min or more of consecutive zero counts were excluded from each individual's recording [22, 23]. Physical activity data were included for further analysis if the subject had accumulated a minimum of 8 h of activity data per day for at least 2 weekdays and 1 weekend day [15]. Total daily physical activity was calculated as the total number of counts divided by the registered time (counts/min). The time spent in light physical activity (2–3 METs), moderate physical activity (3–6 METs) and vigorous PA (>6 METs) was calculated based upon cut-offs of 2,000 and 4,000 counts per minute, respectively [24–26]. The time spent in at least moderate-intensity physical activity (>3 METs) was calculated as the sum of time spent in moderate and in MVPA. We further divided physical activity levels as physical inactivity (sedentary activity) and physical activity (light + moderate + vigorous activity). Each minute over the specific cutoff was summarised in the corresponding intensity level group. A standardised questionnaire was used to assess physical activity-related behavior that included information on being engaged in any athletic training, the socioeconomic state of the family, hours of watching TV or being on the internet (screen time), etc. Sleeping time of the subjects was measured with a questionnaire on an ordinary scale: 1, <7 h; 2, 7 or 8 h; 3, 9 or 10 h; 4, 11 or 12 h; 5, >12 h.

Genotyping of ACE ins/del (I/D) polymorphism

DNA was extracted from blood cells by using the GeneJet Genomic DNA Purification Kit (Fermentas, Lithuania). The ACE gene I/D genotype was determined by polymerase chain reaction (PCR) employing the forward primer 5′-CCCAGGCCGGGGACTCTGTA-3′ and the reverse primer 5′-AGCTCCAGCCCTTAGCTCACCT-3′ (Metabion, Germany). The amplification reaction was performed in total volume of 25 µl containing 50 ng of genomic DNA, 1.2 µl of each primers (10 pmol/µl), 2.5 µl of 2.5 mM dNTP (Solis BioDyne, Estonia), 2.5 µl of 25 mM $MgCl_2$ (Solis BioDyne), 2.5 µl of 10-fold concentrated PCR reaction buffer B [containing 0.8 M Tris–HCl, 0.2 M $(NH_4)_2SO_4$, 0.2 % w/v Tween 20; Solis BioDyne], 0.25 µl Hot FirePol Polymerase (5 U/µl; Solis BioDyne), and ddH_2O. The amplification began with denaturation step at 95 °C for 15 min, followed by 34 cycles of denaturation at 95 °C for 40 s, annealing at 65 °C for 40 s, and extension at 72 °C for 40 s, the final extension step occurred at 72 °C for 7 min. The amplification products (461 and 174 bp) were electrophorised on ethidium bromide staining using agarose gel and visualised under UV light. Genotype I/I was determined as one band at 461 bp, genotype D/D as one band at 174 bp, and two bands at 461 and 174 bp corresponded to the heterozygotic genotype I/D.

Statistical analysis

SPSS v.17.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Descriptive statistics are presented as mean ± SD. All variables were checked for normality of distribution before the analysis. Vigorous physical activity was logarithmically transformed to normalise its distribution. Differences in mean values between different genotypes were assessed using one-way ANOVA. A backward linear regression was used to determine the parameters (body compositional, lifestyle, fitness) that were significantly related to physical activity parameters. Univariate general linear modelling was used to determine the genotype effect on different parameters of physical activity. Those parameters selected by backward linear regression were entered as covariates one by one according to the significance level. LSD post hoc analysis was used to test the differences between the genotypes where a significant genotype effect on dependent variable was seen. There was no solid assumption to collapse two genotype groups to form an allele carrier group based on previous literature (e.g. [6]). Therefore, genotypes were compared both separately and against opposite allele carriers to facilitate clearance and generalization. The statistical significance was set at $p < 0.05$.

Results

The descriptive parameters of the subjects are presented in Table 1. Only body fat mass value showed a statistically significant difference between ACE II and ID genotypes.

Table 1 Descriptive characteristics of the subjects according to ACE (I/D) genotype

Variables	ACE I/D polymorphisms		
	II ($n = 67$)	ID ($n = 114$)	DD ($n = 80$)
Age (years)	12.17 ± 0.72	12.96 ± 0.80	12.08 ± 0.74
Height (cm)	153.9 ± 8.0	154.4 ± 8.4	155.3 ± 8.3
Weight (kg)	46.0 ± 11.2	48.6 ± 14.8	48.4 ± 14.3
BMI (kg/m^2)	19.2 ± 3.3	20.1 ± 4.8	19.8 ± 4.3
Body fat mass (kg)	9.5 ± 6.5	12.7 ± 9.9*	11.8 ± 9.2
Body fat free mass (kg)	33.4 ± 6.1	33.4 ± 6.4	33.9 ± 6.6
CVF (W/kg)	2.94 ± 0.59	2.87 ± 0.66	2.91 ± 0.67

CVF Cardiovascular fitness

* Significantly different from II group; $p < 0.05$

Table 2 Total physical activity and different physical activity categories according to ACE genotype and allele frequences

	ACE genotype				
	II ($n = 67$)	ID ($n = 114$)	DD ($n = 80$)	I allele ($n = 181$)	D allele ($n = 194$)
Average PA (counts/min)	480 ± 151	496 ± 150	524 ± 145	490 ± 150	508 ± 148
Sedentary (min/day)	403 ± 79	412 ± 74	391 ± 52[#]	409 ± 75[a]	403 ± 66
Light PA (min/day)	300 ± 68	316 ± 56	321 ± 59[*]	310 ± 61	318 ± 57[b]
Moderate PA (min/day)	45 ± 22	48 ± 20	49 ± 19	47 ± 21	48 ± 20
Vigorous PA (min/day)	11 ± 10	12 ± 11	13 ± 13	11 ± 11	12 ± 12
MVPA (min/day)	56 ± 28	59 ± 27	62 ± 28	58 ± 27	60 ± 28
Total PA (min/day)	354 ± 9	375 ± 7	382 ± 8*	367 ± 5	378 ± 5[b]

Total PA is the sum of light, moderate and vigorous activities

PA physical activity

* Significantly different from II group; $p < 0.05$

[#] Significantly different from ID group; $p < 0.05$

[a] Significantly different from DD group; $p < 0.05$

[b] Significantly different from II group; $p < 0.05$

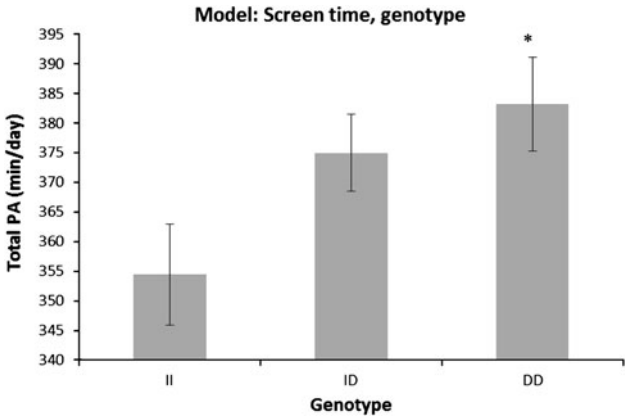

Fig. 1 Associations with ACE I/D polymorphism and total physical activity (PA) levels, with screen time as covariate. Total physical activity level is the sum of light, moderate and vigorous physical activity levels. *Significantly different from II genotype ($p < 0.05$)

The frequencies of ACE I/D polymorphism were in the Hardy–Weinberg equilibrium ($\chi^2 = 3.78$, $p > 0.05$). The distribution of physical activity levels and total physical activity between ACE I/D genotypes are presented in Table 2. Sedentary behaviour level was significantly lower in DD subjects compared to I allele carriers ($p = 0.038$). Both light and total physical activity levels were highest in DD subjects and lowest in II subjects.

No significant relationships between ACE I/D polymorphism and MVPA activities were found. However, the model with three genotype groups revealed a trendline effects on light physical activity ($F_{2,256} = 2.49$; $p = 0.085$; $\eta^2 = 0.015$) and total physical activity ($F_{2,256} = 2{,}979$; $p = 0.053$; $\eta^2 = 0.023$) where subjects with II genotype exhibited the least activity. Combining D allele carriers resulted in a significant main effect on total physical activity ($F_{1,256} = 5{,}453$; $p = 0.020$; $\eta^2 = 0.021$). However, I allele carriers were significantly more sedentary compared to subjects with DD genotype ($F_{1,256} = 3.87$; $p = 0.049$; $\eta^2 = 0.015$). The most influential factors, fat mass ($p < 0.001$) and screen time ($p < 0.033$) were selected by backward regression analysis and were entered into the univariate general linear model analysis as covariates. All the above-mentioned genotype effects weakened when accounting for fat mass (data not shown). However, adding screen time as a covariate accentuated the effect of ACE I/D polymorphism on total physical activity levels ($F_{2,256} = 3{,}326$; $p = 0.041$; $\eta^2 = 0.025$; Fig. 1) with DD genotype subjects having the highest total physical activity levels. Accounting for screen time, the effect of the ACE genotype on light physical activity was also significant, but only when combining ID and DD genotype against II genotype: D allele carriers exhibited higher physical activity ($F_{1,256} = 4{,}710$; $p = 0.031$; $\eta^2 = 0.20$).

Heterozygous subjects differed significantly from homozygous subjects by higher sedentary activity while the total physical activity was not proportionally lowered. As they had also significantly higher body fat mass amounts (Table 1), we hypothesised that they might sleep less compared to homozygous subjects, as shorter sleep time may be associated with adiposity indicators [36]. The analysis confirmed this hypothesis and indicated that subjects with ID genotype were sleeping significantly less time compared to II and DD genotype [2.50 (0.50); 2.33 (0.54) and 2.52 (0.55) for II, ID, and DD genotypes, respectively].

Discussion

The current study is one of the few in the literature to investigate genotype effects on habitual physical activity in

12-year-old boys. We did not find any ACE genotype or allele effect on higher physical activity levels (i.e. moderate and vigorous physical activity), which are considered as the most important activity levels related to cardiovascular health risks in children. However, carrying the D allele of the ACE gene had a main effect on total physical activity with screen time as covariate. In contrast, carrying the I allele was instead related to sedentary behaviour. These results indicate that ACE genotype groups cannot always be combined to a priori allele carrier groups (e.g. I allele against D/D genotype).

ACE I/D polymorphism is a functional polymorphism that has direct influence on plasma ACE level [6], which promotes growth for cardiac myocytes and fibroblasts. DD homozygotes have been shown to have higher angiotensin II levels which may elicit vasoconstriction and consequently decreased oxygen and substrate delivery to muscles, that further may result in decreased energy metabolism [27]. Considering those effects, numerous studies in the literature have investigated this genotype effect on several aspects of physical performance (for review, see [8]). However, there are fewer studies that have investigated genotype effect on habitual physical activity, as there may be a link between physical activity and performance. Recent studies have shown that not only is MVPA level decreasing (Nader et al. [1]) but sedentary behaviour is also increasing, and spending large amounts on MVPA activities does not necessarily mean that sedentary activities are decreased [18, 28].

In the current study, an independent trendline gene effect was found in light and total physical activity levels ($p = 0.085$ and $p = 0.065$, respectively). However, environmental factors are important determinants of habitual physical activity [4, 29], therefore we studied additionally several covariates that might have significant effects on physical activity. Although a covariate itself may have a genetic influence, it also has a large environmental effect on physical activity and therefore has to be taken into account. Screen time—sitting in front of the TV or computer—is a large contributor ($p < 0.05$) for physical activity and has probably more direct influence on sedentary behaviour (friends, interesting shows, etc., probably also genetic influence) compared to the ACE gene and therefore should be taken into account if studying the gene effect, as the subjects with the I allele spent significantly more time in sedentary activities compared to the DD genotype, with screen time as a covariate. On the other hand, D allele carriers spent more time in light activities compared to the II genotype. Recently, it has been argued that sedentary behaviour per se may not represent the low end of the physical activity continuum, but may represent a different behavioural paradigm [8, 30]. Therefore, in this study, we further divided physical activity into sedentary (physical inactivity) and total physical activity (the sum of light, moderate and vigorous physical activity). In this case, ACE I/D polymorphism was associated with total physical activity levels ($p = 0.041$), with screen time as covariate. Furthermore, a main D allele effect was found in total physical activity levels ($p = 0.031$). According to these results, it can be concluded that carrying a D allele was related to a more "favorable" physical activity profile. Recent studies in adults have found that subjects with the II genotype may perform better in endurance-type activities [8]. In this regard, our hypothesis that the better predisposition to endurance activities would instead result in higher physical activity levels was rejected. However, there are some data in the literature that might explain this finding. The D allele has been found to be related to left ventricular mass [31] which has, in turn, been related to higher performance. This might also favor higher physical activity levels of the subjects of a particular age, since their aerobic capacity is not yet fully developed. However, further studies are needed in this area to clarify what would be the mechanism by which ACE influences physical activity. Our results are similar to the results of Lauderdale et al. [3] who also indicated that the majority of the effect on physical activity was related to environmental not genetic factors. In our study, the gene effect also became significant if environmental factors were taken into account. Although Lauderdale et al.'s [3] result was related to higher physical activity levels, it can be suggested that the environmental effect is also similar to lower physical activities.

Our study differs from Winnicki et al.'s [14] study, in which it was found that ACE I/D polymorphism might be a specific genetic factor associated with physical activity in mild hypertensive subjects. Those differences can be explained by the fact that subjects in Winnicki et al.'s [14] study represented a clinical population (age 18–45 years) which might have had an influence compared to the population-based sample in our study. Furthermore, Winnicki et al. [14] only reported the total number of II or DD genotypes within sedentary mild hypertensive subjects. This result, however, does not allow us to conclude how much time they actually spent in sedentary activities. Furthermore, the sedentary classification in their study was less clearly defined and included subjects who did not perform regularly in any sports activity, which further does not allow differentiating between sedentary or light activity levels. As light physical activity frequently occurs in daily life, it is more difficult to accurately quantify light physical activity than MVPA when using PA questionnaires, especially in young subjects [32].

We did not find any gene effect on MVPA activity levels. This finding might be explained by the fact that higher physical activity levels may be related to

participation in sports training. However, in our study, the participation in sports training was not associated with physical activity levels ($p > 0.146$). Moreover, there was a lack of power (<0.431) to test this hypothesis due to the relatively small amount of time the subjects were in MVPA. A similar finding was observed by Sarzynski et al. [15], who found that MVPA levels were not related to ACE I/D genotype. On the other hand, taking part in sports training might be significantly related to the demand of the parents or by friends' participation, in particular sports training that finally results in child participation. Unfortunately, we were not able to control the influence of parents or close friends on sports training participation.

Physical activity and sedentary activity independently predict chronic disease risk and mortality, and should, therefore, be considered as separate constructs [28]. Light physical activity involves lower energy expenditure than MVPA, but higher energy expenditure than inactivity. Therefore, the increase in daily light physical activity might have a protective effect against increases in fat mass due to higher energy expenditure. In general, it seems that the subjects in our study were more sedentary, but slightly more in MVPA compared to similar age subjects in different studies. The average MVPA activities in the current study averaged 59 ± 26 min, with more than half of the subjects exceeding the recommended physical activity (60 min/day) levels. A recent meta-analysis of 14 different studies suggested that accumulating more than 35 min in MVPA will result in more favourable metabolic health regardless of the time being sedentary [28]. Although research to date is limited regarding the association between light physical activity and adiposity during childhood, recent studies have demonstrated a negative association between time in light physical activity and adiposity indicators [33, 34]. In contrast, time spent in sedentary activities was unrelated to health risks after adjusting for MVPA [28]. However, mechanisms underlying the health outcomes associated with sedentary behaviour and physical activity may be linked to different physiological pathways [35]. Therefore, it should be considered that in studying the effects of physical activity on health not only moderate to vigorous activities are important, but the full range of energy expenditure (activity range) should be taken into account [18]. Considering those effects, our findings are important in the field of physical activity and health. As we were able to demonstrate ACE genotype effect on the amount of sedentary (physical inactivity), light and total physical activity, the prevention strategies focused at this period of life should take into account that a sedentary lifestyle in children is related to gene effects.

Surprisingly, subjects with the ID genotype had significantly higher body fat mass values compared to subjects with the II genotype ($p < 0.05$). This anomaly led us to hypothesise whether this can be related to their sleep time, although this was not the main focus of the study. Having less sleep has been found to be related to higher body fat values [36]. As we had sleep duration data available, we could check the hypothesis. We found that subjects with the ID genotype had less sleep time compared to homozygous subjects ($p < 0.05$). Therefore, we could speculate that the effect of the I allele on sedentary behaviour can mainly be found in ID subjects and is probably linked to other environmental or genetic factors (i.e. another genotype that is in linkage disequilibrium with ACE).

The strengths of this study are: (1) the use of a relatively young cohort of boys, that allowed to eliminate several possible cofounders like sex, variations in age, etc.; and (2) several environmental covariates (training status, screen time, aerobic fitness, body composition) were taken into account when investigating gene effects on physical activity level; however, the possible epigenetic regulation has yet to be determined. In contrast, the use of a 10-min cut-off might also have some influence on the results, because this method probably underestimates sedentary time. However, a similar cut-off has been used in recent studies with special targets for sedentary behaviour [22, 23]. There is also a possibility that ACE I/D polymorphism may be in strong linkage disequilibrium with other genes and the physical activity phenotype can be an expression of complex genetic changes. Indeed, there are several other genetic alterations associated with athletic performance (such as Arg577STOP in ACTN3 gene) [8], which could possibly affect habitual physical activity. It has to be further taken into account that significant covariates in the models—screen-time, and fat mass—themselves are a function of genetic and environmental factors, but the investigation of the effects on covariates was beyond of the scope of this study. We did not use the Bonferroni correction because our study was hypothesis-based and therefore we presented the results with uncorrected p values.

In conclusion, the ACE I/D polymorphism is associated with different levels of physical activity in healthy boys, accounting for the confounding factor of screen time. Our findings emphasise the facilitating role of the D allele on total physical activity.

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Conflict of interest The authors of this manuscript have no conflicts of interest.

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