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Author(s): Haapala, Eero A.; Gao, Ying; Rantalainen, Timo; Finni, Taija

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We investigated the associations of age, sex, body size, body composition, and maturity with measures of physical activity (PA) intensity in children. PA intensity was assessed using $\dot{V}O_2$ as % of $\dot{V}O_{2reserve}$ or $\dot{V}O_{2}$ at ventilatory threshold (VT), muscle activity measured by textile electromyography, mean amplitude deviation (MAD) measured by accelerometry, and metabolic equivalent of task (MET) during laboratory activities. Age, stature, and skeletal muscle mass were inversely associated with VO2 as % of VO2reserve and VO2 as % of VO2 at VT, during walking or running on a treadmill for 4, 6, and 8 km/h (Spearman r=-0.645 to -0.358, p<0.05). Age was inversely associated with MAD during walking on treadmill for 4 km/h (r=-0.541, p<0.05) and positively associated with MAD during running on a treadmill for 8 km/h, playing hopscotch, and during self-paced running (r=0.368 to 0.478, p<0.05). Fat mass was positively associated with VO2 as % of VO2reserve and VO2 as % of VO2 at VT and waist circumference was positively associated with VO2 as a % of VO_{2reserve} and muscle activity during stair climbing (r=0.416 to 0.519, p<0.05). Fixed accelerometry cut-offs used to define PA intensities should be adjusted for age, sex, body size, and body composition. **Key words:** child; physical activity; accelerometry; electromyography; body composition **Disclosure statement:** No potential conflict of interest was reported by the authors. Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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INTRODUCTION

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Accelerometry has become the preferred device-based method to assess volume and intensity of habitual physical activity (PA) ¹. Higher levels of moderate-to-vigorous intensity physical activity (MVPA) and vigorous PA (VPA) assessed by accelerometry have been consistently associated with lower levels of adiposity, cardiometabolic risk, arterial stiffness, and higher cardiorespiratory fitness in children and adolescents ^{1,2}. Cut-offs defining light PA, moderate PA, and vigorous PA in children and adolescents have been created using specific calibration activities ^{3–5}. These calibration activities have been used to investigate the point of acceleration magnitude separating e.g. slow walking as light PA from brisk walking and climbing up and down the stairs as moderate PA ^{3–5}. Although some of these calibration studies have measured oxygen uptake (VO₂) during the calibration activities, PA intensity cut-offs have been mainly created using subjective criteria based on calibration activities rather than physiological responses ^{3–5}. Furthermore, converting accelerometry metrics to metabolic equivalents of tasks (METs) and utilising commonly used MET-based cut-offs for PA intensities have been suggested to improve comparability between studies ⁶. However, physiological rationale for the usage of METs in the assessment of PA intensity is lacking ⁷ and fixed MET-based cut-offs have been found to underestimate PA intensity and volume in unfit and obese adults⁸ and to misclassify PA intensity in children 9. Growth and maturation are dominant biological processes during childhood and adolescence characterised by increased body size, sexually dimorphic changes in body composition, and morphological and functional changes in cardiorespiratory, neuromuscular, and metabolic systems ^{10,11}. Exercise capacity also increases with increasing age and advancing maturity ¹⁰.

Nevertheless, none of the previous calibration studies have taken body size, maturation, or body composition into account in the proposed cut-offs ^{3–5,12}. Furthermore, previous large scale PA studies in paediatric populations have used the same fixed accelerometry cut-offs for the assessment of PA intensity for children and adolescents with age of the participants varying from 3 to 18 years ^{13–17}. However, because of growth and maturation and accompanying changes in locomotor economy ^{18,19}, using the same cut-offs in children and adolescent with different ages, body sizes, and body compositions may lead to large errors in the estimation of PA prevalence and the associations of PA with health and wellbeing.

Nevertheless, some evidence also suggests that the same accelerometer cut-offs could be applied for adolescents and adults ²⁰. Furthermore, some studies suggest that muscle activity measured by electromyography (EMG) could provide a direct and useful measure of PA intensity in children ²¹, but the knowledge on the associations of age, sex, body size, body composition, and maturity with muscle activity in different PA intensity calibration activities is limited. Therefore, research on the role of age, growth, and maturation on the PA intensity during the calibration activities is warranted.

Available and commonly used acceleration magnitude cut-offs utilised to define PA intensity in children and youth^{3–5,12} are based on several different methods and calibration tasks. However, these fixed PA intensity cut-offs provide absolute values which are used to define light PA, moderate PA, and vigorous PA in children and adolescents without consideration whether proposed PA intensity cut-offs describe the same intensity among children with different body sizes and body compositions or among different age- and maturation groups. Therefore, we investigated the associations of age, sex, body size, body composition, estimated years from the peak height velocity (YPHV), and pubertal status with acceleration magnitude and MET-based PA intensity in children. We further investigated whether age,

sex, body size, body composition, estimated years from the peak height velocity (YPHV), and pubertal status were associated with individualised measures of PA intensity defined using $\dot{V}O_{2reserve}$, ventilatory threshold (VT), and muscle activity.

METHODS

Participants

This study was based on the laboratory phase of the Children's Physical Activity Spectrum (CHIPASE) study ²². A total of 35 children (21 girls, 14 boys) aged 7–11 years were recruited from local schools by leaflets and word of mouth advertisement to participate in the study. Volunteering children were accepted into to the study sample in the order of enrolment. The applicability of the children was checked by the research staff and children were included if they were apparently healthy and were able to perform the physical activities at moderate and vigorous intensities. Children with chronic conditions or disabilities were excluded from the study. The number of participants in the current data analyses varied from 27 to 35 participants with acceptable data quality. Most missing data was from the activity where the participants were asked to run around an indoor track at self-paced speed (27 participants with acceptable METs data). The study protocol was approved by the Ethics Committee of the University of Jyväskylä. All children gave their assent and their parents/caregivers gave their written informed consents. The study was conducted in agreement with the Declaration of Helsinki.

Based on the main research question of the CHIPASE Study, a sample size of 30 was estimated to provide sufficient statistical power for differentiating METs between sitting (1.33 ± 0.24) and standing (1.59 ± 0.37) based on the data of Mansoubi et al.²³ with 80% power and 5% α -error level.

Study protocol

The participants visited the laboratory on three occasions. At the first visit, research staff explained the research protocol to children and their parents. They were also familiarised to the laboratory environment and measurement equipment. At the second visit, children arrived at the laboratory in the morning after 10-12 hour overnight fast for assessment of anthropometrics, body composition, and resting $\dot{V}O_2$. At the third visit, children were asked to perform following activities for 4.5 minutes in a random order interspersed with 1-minute rest: sitting quietly, sitting while playing a mobile game, standing quietly, standing while playing a mobile game, playing hopscotch, walking up and down the stairs, and walking or running on a treadmill at 4, 6, and 8km/h. They were also asked to walk and run around an indoor track at self-chosen speed for 4.5 minutes. At the end of the third visit, children performed maximal cardiopulmonary exercise test on a bicycle ergometer.

Assessments

Body size and body composition

Stature was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body mass (BM), skeletal muscle mass (SMM), fat mass (FM), fat free mass, and body fat percentage were measured by InBody 770 bioelectrical impedance device (Biospace Ltd., Seoul, Korea). Body mass index (BMI) was calculated by dividing body weight with body height squared and body mass index standard deviation score (BMI-SDS) was computed using the Finnish references ²⁴. Waist circumference (WC) was measured to the nearest 0.1 cm using a unstrechable measuring tape at mid-distance between the top of the iliac crest and the bottom

of the rib cage. Hip circumference (HC) was measured at the widest circumference over the great trochanter.

Years from peak height velocity and pubertal status

Years from peak height velocity and pubertal status (YPHV) was estimated using a sexspecific formula described by Mirwald et al. ²⁵. Pubertal status was assessed according to self-reported genital development in boys and breast development in girls on the basis of the five-stage criteria described by Tanner ²⁶. We defined those at Stage I as pre-pubertal and those at Stage II as those who had entered puberty.

Oxygen uptake

Mobile metabolic cart (Oxycon mobile, CareFusion Corp, USA) was calibrated and dead space was adjusted to 78 mL for the petite size of the face mask following the manufacturer's recommendations. $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$) and respiratory exchange ratio (RER) were collected breath by breath and computed in non-overlapping 1 second epoch lengths. Resting $\dot{V}O_2$ was determined as the mean value between the 15th and 25th minute of 30 minutes of supine rest when the steady state was reached. When steady stated was not observed between 15th and 25th minute, the steady state was visually selected for further analysis. In physical activities, $\dot{V}O_2$ was averaged over 2 minutes from the 3rd and 4th minutes of each task when plateau in $\dot{V}O_2$ and $\dot{V}CO_2$ was observed ²². $\dot{V}O_2$ reserve as a percentage of $\dot{V}O_{2peak}$ during different physical activities was calculated as ($\dot{V}O_2$ during PA task / ($\dot{V}O_{2peak}$ - $\dot{V}O_2$ during rest)) x 100. $\dot{V}O_2$ at VT was determined individually by two exercise physiologists using modified V-slope method and any disagreements were solved by these two exercise physiologists. $\dot{V}O_2$ at VT was verified utilising the equivalents for \dot{V}_E / $\dot{V}CO_2$ and \dot{V}_E / $\dot{V}O_2$.

Accelerometry

Movement was measured by triaxial accelerometer (X6-1a, Gulf Coast Data Concepts Inc., Waveland, USA). We used raw acceleration data in actual g-units, with range up to 6g, 16-bit A/D conversion, and sampling at 40 Hz. The resultant acceleration of the triaxial accelerometer signal was calculated from $\sqrt{x^2 + y^2 + z^2}$, where x, y and z are the measurement sample of the raw acceleration signal in x-, y-, and z-directions. The X6-1a accelerometer has been shown to produce congruent results with the ActiGraph GT3X accelerometer in children ²⁷. Mean amplitude deviation (MAD) was calculated from the resultant acceleration in non-overlapping 1 s epoch. MAD was calculated as the mean distance of data points about the mean ($\frac{1}{n}\sum_{i=1}^{n}|r_{i}-\vec{r}|$ where n is the number of samples in the epoch, r_{i} is the i^{th} resultant sample within the epoch and r^{-} is the mean resultant value of the epoch)^{20,28}. The mean of the 1 s MAD values (g) calculated in 2 minute epochs for each activity and in 10 minute epoch for lying down are reported as the outcomes.

Textile electromyography

Textile EMG electrodes embedded into elastic garments were used to assess muscle activity from the quadriceps and the hamstring muscles and has been described in detail previously 22 . Four different sizes of EMG shorts (120, 130, 140, and 150 cm) with zippers located at the inner sides of short legs and adhesive elastic band in the hem ensured proper fit in every child. The conductive area of the electrodes over the muscle bellies of the left and the right quadriceps was 9×2 cm² (length × width) in all short sizes, while the corresponding sizes for the hamstring muscles were 6×2 cm² in sizes of 120, 130, and 140 cm and 6.5×2 cm² in size of 150 cm. The conductive area of the reference electrodes was 11×2 cm², and they were located longitudinally over the iliotibial band. Water or electrode gel (Parker

Laboratories Inc., Fairfield, NJ, USA) was used on the electrode surfaces to minimize the skin-electrode impedance.

In the signal analysis, EMG data were identified from different activities in the certain time windows simultaneously according to the steady state in respiratory gases. Individual EMG activities were normalised channel by channel to EMG amplitude measured during self-paced walking. The normalised EMG data were averaged for quadriceps from right and left side and hamstring muscles from right and left side, then the mean amplitude of the average normalised data was computed as the intensity of muscle activity level for each activity.

Statistical methods

Basic characteristics between girls and boys were compared using Student's t-test for normally distributed continuous variables and Mann-Whitney U-test for skewed continuous variables and χ^2 - test for categorical variables. We investigated the correlations of age, stature, weight, BMI, BMI-SDS, FM, SMM, YPHV, and pubertal status to $\dot{V}O2$ as a % of $\dot{V}O2$ at VT, MAD, METs, and muscle activity during different activities using Spearman correlation coefficients. Differences in $\dot{V}O2$ as a % of $\dot{V}O2$ reserve, $\dot{V}O2$ as a % of $\dot{V}O2$ at VT, MAD, METs, and muscle activity between girls and boys and between prepubertal and those who had entered clinical puberty were investigated using Kruskal-Wallis test. Student's t-test, the Mann-Whitney U-test, the $\chi 2$ test, and the Kruskal-Wallis tests were performed using the SPSS Statistics, Version 23.0 (IBM Corp., Armonk, NY, USA). The data were visualised and Spearman correlations were performed by the GraphPad Prism, version 8.0.2 (Graph Pad Software, Inc., San Diego, CA, USA). The data were analysed using non-parametric tests because the assumptions to use parametric were not met for some variables. Because of the large number of statistical analyses, we utilised

Benjamini-Hochberg false discovery rate (FDR) procedure and Bonferroni correction for multiple testing in the correlation analyses. Correction for multiple testing was performed for each PA intensity indicator. We used false discovery rate of 0.1 in the Benjamini-Hochberg procedure. Furthermore, with the Bonferroni correction the threshold for statistical significance was computed dividing the p-value of 0.05 by the number of variables used in each analysis resulting in the corrected critical value of 0.006. **RESULTS** Basic characteristics and the associations between the measures of age, body size, composition, and maturation Girls were lighter, had lower BMI, and had less FM and SMM than boys (Table 1). Girls were also closer to their estimated PHV than boys (Table 1). Age and the measures of body size and body composition were strongly and positively correlated (Supplementary Table). YPHV was positively correlated to age, stature, SMM, and HC. Children who had entered to puberty were taller (mean=143.9 (SD=8.2) vs. 135.3 (8.9) cm) and heavier (37.1 (6.5) vs. 30.7 (6.3) kg), and had more SMM (16.2 (2.4) vs. 13.1 (2.7) kg) than pre-pubertal children (all p<0.05). Associations of sex, age, body size, and maturation with physical activity intensity Girls vs. boys Girls operated at higher intensity relative to their VO_{2reserve} during running on a treadmill for 8 km/h and during self-paced walking than boys (Figure 1). Girls had lower muscle activity (i.e. lower EMG signal normalised for EMG signal during self-paced walking) during walking on a treadmill for 6 km/h, walking up and down the stairs, and during playing hopscotch. Girls also had higher MET during running on a treadmill for 8 km/h and during

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playing hopscotch than boys. However, girls had lower MAD during walking up and down 249 the stairs than boys. 250 251 252 Age Age was inversely associated with VO2 as a % of VO2_{reserve} and VO2 as a % of VO2 at VT, 253 during walking or running on a treadmill for 4, 6, and 8 km/h (Table 2). Age was also 254 inversely associated with MAD during walking on a treadmill for 4 km/h and positively 255 associated with MAD during running on a treadmill for 8 km/h and during self-paced 256 257 running. The effect of correction for multiple testing using Benjamini-Hochberg FDR and Bonferroni is demonstrated in Table 2. 258 259 Body size and body composition 260 Stature and SMM were inversely associated with VO2 as a % of VO2reserve and VO2 as a % of 261 VO2 at VT, during walking or running on a treadmill for 4, 6, and 8 km/h (Table 2). 262 Furthermore, FM was positively associated with VO2 as a % of VO2reserve and VO2 as a % of 263 VO2 at VT and WC with VO2 as a % of VO₂reserve and muscle activity during climbing up 264 and down the stairs. The effect of correction for multiple testing using Benjamini-Hochberg 265 FDR and Bonferroni is demonstrated in Table 2. 266 267 Maturity 268 Children who had entered puberty operated at lower intensity relative to their VT and they 269 had lower MET-values during walking on a treadmill for 4 km/h than their pre-pubertal peers 270 (Figure 2). YPHV was inversely associated with VO2 as a % of VO2 at VT during walking 271 or running on a treadmill for 4, 6, and 8 km/h and MAD during walking on a treadmill for 4 272 km/h but positively with MAD during running on a treadmill for 8 km/h (Table 2). The effect 273

of correction for multiple testing using Benjamini-Hochberg FDR and Bonferroni is demonstrated in Table 2.

There were also other but inconsistent associations between the measures of age, maturation, body size, body composition, and the measures of PA intensity (Table 2).

DISCUSSION

We found that older children and those who were taller and had more SMM operated at lower PA intensity level relative to their $\dot{V}O_{2reserve}$ and VT during walking or running on a treadmill for 4, 6, and 8 km/h. We also found that children with higher levels of adiposity operated at higher intensity level relative to their $\dot{V}O_{2reserve}$ and VT during climbing up and down the stairs than leaner children. Furthermore, boys operated at lower intensity level relative to their $\dot{V}O_{2reserve}$ during running on a treadmill and self-paced walking than girls, but the sex-differences in METs and muscle activity were heterogenous. The associations of age, body size, and body composition with PA intensity estimated by MAD or METs were inconsistent and weak, suggesting that they were not able to capture physiological PA intensity. Finally, 79% and 37% of statistically significant associations remained significant after Benjamini-Hochberg FDR or Bonferroni corrections for multiple testing, respectively. The most robust associations even after corrections for multiple testing were those of stature and SMM with $\dot{V}O_{2reserve}$ and VT during walking or running on a treadmill for 4, 6, and 8 km/h.

Previous calibration studies have defined light PA as walking on a treadmill for ~3–4 km/h, MPA as stair climbing and walking on a treadmill for ~5–6 km/h, and VPA as running on a treadmill for ~6.5–8 km/h ^{13–15}. We found that older children and those who were taller and had more SMM and who were more mature performed constant speed activities on treadmill

at lower physiological intensity level than other children. Nevertheless, we found no marked differences in other more self-paced tasks, such as playing hopscotch and self-paced walking and running, used to assess light PA, moderate PA, or vigorous PA in children. For example, age or stature were not associated with $\dot{V}O_{2reserve}$ and VT during climbing up and down the stairs, which has been previously considered moderate intensity PA. Reasons for our findings may be that constant treadmill speeds require more effort from younger and smaller children than from older and taller children because of lower required step frequency ¹⁹, better walking and running economy ^{18,29} and ability to store elastic energy ³⁰, and increased cardiorespiratory capacity ³¹ with increasing age and stature. Because climbing up and down the stairs and other activities in our study were performed at a self-paced speed, it is possible that children adapted their effort to fit their fitness level. Nevertheless, we found a positive association between adiposity and PA intensity during climbing up and down the stairs that could be due to excess inert mass that must be carried during climbing up and down the stairs ³².

We found no consistent associations of age and stature with muscle activity during treadmill activities suggesting that differences in muscle activity during constant speed treadmill walking and running are not a factor of body size or body composition children. These results correspond to our earlier observations that muscle strength is not associated with PA intensity during treadmill walking and running (Haapala et al. unpublished observation). Furthermore, a large interindividual variability in muscle activity in different activities in children ²², may explain our observations. However, we observed that boys, older children, and who were taller, heavier, and had more FM had higher muscle activity during playing hopscotch. Furthermore, children with higher WC and HC had higher muscle activity during climbing up and down the stairs and playing hopscotch. Adults have been found to exhibit larger muscle

activity especially during eccentric activities ³⁰ and playing hopscotch and climbing up and down the stairs include strong eccentric phases. Therefore, it is possible that the observed associations reflect increased muscle activity in boys and older, taller, and heavier children during eccentric phase.

Despite significant associations between body size and body composition with $\dot{V}O_{2reserve}$ and VT, we found inconsistent and mixed associations of body size and composition with PA intensity measured by MAD and METs. Interestingly, we observed higher MAD in older and taller children during running on a treadmill and during self-paced running, although older and taller children had lower $\dot{V}O_{2reserve}$ and VT during those activities. Therefore, these results suggest that MAD overestimate PA intensity especially in higher intensity activities among older and taller children. However, while the exact reason for these findings is unclear, it is possible that older and taller children modify their walking and running styles between slower and faster treadmill speeds as some previous evidence suggests that there are natural differences in walking and running mechanics between children and adolescents with varying ages 33 . Finally, in line with previous studies in adults 8,34 , METs were not able to differentiate physiological differences in PA intensity in children with different body sizes and compositions.

The strengths of the present study include a valid and simultaneous assessment of different measures of PA intensity such as $\dot{V}O_2$, muscle activity by EMG, and accelerometry during different activities. The assessment of different PA intensity indicators allowed us to form more complete picture of PA intensity during the activities. We also assessed of $\dot{V}O_{2peak}$ and VT, body size, and body composition using valid methodology. Nevertheless, we had some missing data due to malfunction of the devices or poor data quality in some tasks which may

influence the results. $\dot{V}O_{2peak}$ and VT were assessed during a maximal cycle ergometer test and $\dot{V}O_{2peak}$ was adjusted using the data from treadmill running or self-paced running if higher $\dot{V}O_2$ was observed during those tasks. Therefore, it is possible that we have underestimated true $\dot{V}O_{2max}$ in some participants and this may have had a minor effect on $\dot{V}O_{2reserve}$ estimation. Furthermore, we estimated APHV and assessed pubertal status using self-reports instead of measuring circulating sex steroids or using clinical examination of secondary sex-characteristics. In addition, the increasing error in the estimation of APHV with increasing time to PHV could have an effect on the estimated maturity status in our sample of relatively young children. Further studies on the effect of maturation on PA intensity utilising more accurate methods, such as skeletal age, in the assessment of maturity are warranted. Because of relatively small sample size, we were not able to study whether age or maturity groups modified the observed associations of body size and composition with PA intensity in different tasks. Finally, the relatively large number of analyses increases the possibility that some statistically significant associations were observed by chance.

CONCLUSION

In conclusion, we found inverse association of age, stature, and SMM with PA intensity defined using $\dot{V}O_{2reserve}$ and VT. However, MADs and METs did not reliably capture these associations and our results suggest that PA intensity estimated by MAD may overestimate PA intensity in older and taller children. Therefore, our results suggest that studies validating accelerometry or muscle activity cut-offs used to define PA intensities should be adjusted for age, sex, body size, and body composition. Further studies on the role of these adjustments of the prevalence of children meeting the PA recommendations are warranted.

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Figure legends

Figure 1. Differences in the measures of physical activity intensity between girls and boys.

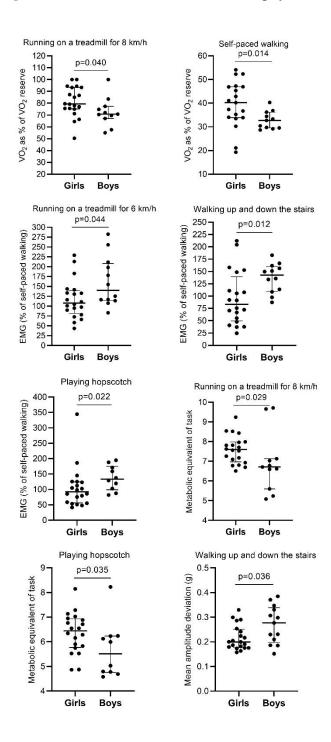


Figure 2. Differences in the measures of physical activity intensity between prepubertal and pubertal children.

