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Crotti, M, Foweather, L, Rudd, JR, Hurter, L, Schwarz, S and Boddy, LM

Development of raw acceleration cut-points for wrist and hip accelerometers to assess sedentary behaviour and physical activity in 5-7 year old children

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- Development of raw acceleration cut-points for wrist and hip accelerometers to assess
 sedentary behaviour and physical activity in 5-7 year old children
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4 Abstract:

The purpose of the study was to validate sedentary behaviour (SB), moderate-to-vigorous 5 6 physical activity (MVPA) and vigorous physical activity (VPA) accelerometer cut-points for wrist and hip-worn ActiGraph devices in 5-7 year old children. Forty-nine (n=27 girls) 5-7-7 vear-old children were recruited. Participants wore an ActiGraph GT9X accelerometer, 8 9 recording data at 100Hz subsequently downloaded in 1s epochs, on both wrists and the right hip during a standardised protocol (10 tasks ranging from lying to running), and during recess. 10 Cut-points were generated using ROC analysis using direct observation as a criterion reference 11 12 in the cut-point generation group (n=22, 50% girls). Subsequently, cut-points were modified using Confidence intervals equivalency analysis until optimal cut-points were identified. Cut-13 points were then cross-validated using a cross-validation group (n=10, 60% girls). SB cut-14 points were 36mg (Sensitivity(Sn)=79.8%, Specificity(Sp)=56.8%) for non-dominant wrist, 15 39mg (Sn=75.4%, Sp=70.2%) for dominant wrist and 20mg (Sn=78%, Sp=50.1%) for hip. 16 MVPA cut-points were 189mg (Sn=82.6%, Sp=78%) for non-dominant wrist, 181mg 17 (Sn=79.1%, Sp=76%) for dominant wrist and 95mg (Sn=79.3%, Sp=75.6%) for hip. VPA cut-18 points were 536mg (Sn=75.1%, Sp=68.7%) for non-dominant wrist, 534mg (Sn=67.6%, 19 20 Sp=95.6%) for dominant wrist and 325mg (Sn=78.2%, Sp=96.1%) for hip. All accelerometer placements demonstrated adequate levels of accuracy for SB and PA assessment. 21

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Key Words: Accelerometry, validation, raw signal, objective measurement, criterion validity

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29	Authors:
30	Mr. Matteo Crotti ^{1,2*} , Dr. Foweather Lawrence ² , Dr. James R. Rudd ¹ , Mrs. Liezel Hurter ² ,
31	Mr. Sebastian Schwarz ¹ , Dr. Lynne M. Boddy ²
32	Affiliations:
33	1. Department of Sport Studies, Leisure and Nutrition, Liverpool John Moores University,
34	UK
35	2. Physical activity Exchange, Research Institute for Sport and Exercise Science,
36	Liverpool John Moores University. UK
37	Corresponding Author:
38	* Mr Matteo Crotti: No 5 Primrose Hill, Liverpool, L3 2EX, M.Crotti@2016.ljmu.ac.uk
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54 Introduction

Accelerometers are the most widely used devices to assess physical activity (PA) and 55 sedentary behaviours (SB) in children and have proved to be a feasible method to assess 56 57 children on a large scale (1, 2). For many years, hip-worn accelerometers were the preferred devices for PA assessment (3). A major problem with hip-worn devices is poor compliance, 58 59 which has been attributed to discomfort whilst wearing or forgetting to wear the devices after removal (4). However, it was reported that a 24h wear time protocol with hip monitors can lead 60 to high levels of compliance (5). More recently, researchers have used wrist-worn 61 62 accelerometers as they obtain better wear compliance (4, 6) and are suitable for 24-h per day recording, allowing sleep-time assessment (7, 8). A further advantage of wrist-worn 63 accelerometers is that they are more sensitive to upper body movement, considered as a 64 significant component of children's PA (4). 65

Traditionally, accelerometer output was reduced to proprietary units defined as 66 "counts" (9). However, comparing PA and SB estimates across studies that have used different 67 devices brands is problematic because of the brand specific data processing algorithms used 68 (10). Consequently, a methodological harmonisation was recommended involving the use of 69 70 raw acceleration signals rather than counts, regardless of the device brand (11). Raw signals consist of gravitational accelerations assessed at sample frequencies typically above 10Hz. The 71 Euclidean Norm Minus One (ENMO), calculated using the R GGIR package, is emerging as 72 73 the most frequently used metric when processing raw acceleration data generated from the most commonly used triaxial accelerometers (ActiGraph, GENEActiv and Axivity) (12, 13). 74 The use of raw acceleration metrics such as ENMO have the potential to facilitate comparisons 75

76 between different brands and wear sites (4) and to increase researchers' control over data 77 processing. PA and SB intensity cut-points derived for use with ENMO data have been developed for the ActiGraph accelerometers for older children and adults (14, 15). Due to the 78 79 characteristic intermittent nature of the movement behaviours during childhood and in view of the differences in movement dynamics observed in different age groups it is fundamental to 80 create age specific cut-points (16, 17). However, to the best of our knowledge no calibration 81 82 study has established raw acceleration cut-points for ActiGraph devices to assess PA or SB in 5-7 year old children. 83

84 The majority of previous calibration studies have been performed in laboratories and involved equipment such as treadmills or indirect calorimetry that could affect children's 85 movement patterns and gait (18). Concerns have been raised about the ecological validity of 86 87 such settings and it is has been recommended that future calibration studies should involve activities that are representative of free-living PA (19). Additionally, calibration studies should 88 consider accelerometers' limitations in assessing SB based on the absence of or low levels of 89 90 acceleration and distinguishing stationary activities such as standing stationary from SB (15, 20). 91

A further consideration in developing cut-points concerns the statistical techniques used 92 to identify and validate intensity thresholds. Calibration studies have typically used Receiver 93 94 Operating Characteristic (ROC) curve analysis for the calculation of SB and PA intensity cut-95 points from raw accelerometer data (18). Intensity thresholds were typically derived by coding and grouping all the accelerations recorded during the calibration protocol into binary indicator 96 variables (0 or 1) based on the observed or measured activity level (18). However, the 97 98 proportion of data from each activity level (e.g. SB, LPA, MPA and VPA) used in ROC analysis plays a key role in determining PA and SB cut-points and in some case could lead to 99 low accuracy in SB and PA assessment. For example the presence of a high proportion of SB 100

101 acceleration in the ROC analysis dataset could lead to LPA, MPA and VPA cut-points that are too low to accurately classify the behaviour (21). In light of this, alternative statistical 102 procedures that could lead to increased diagnostic accuracy should be evaluated. The use of 103 'pairs' of activity levels in ROC analysis (e.g. SB versus LPA) rather grouped activities (i.e., 104 SB versus LPA, MPA and VPA) has the potential to account for disproportions of data in 105 different activity levels and might lead to improved diagnostic accuracy. However, to date, no 106 107 study has evaluated the diagnostic accuracy of SB and PA cut-points calculated by ROC curve analysis using 'pairs' of activity levels. 108

In view of the gaps in the literature presented above, this study aimed to develop and
validate raw acceleration cut-points for the estimation of SB and PA in 5-7-year-old children
using ActiGraph devices, and to compare different methods of cut-point calculation.

112

113 Methods

114 Design and Participants

The study received institutional research ethics committee approval (17/SLN/004). After school gatekeeper consent was obtained from the headteacher of a single primary school in a metropolitan city in North-West England, parent/carer consent and child assent forms were distributed to potential participants (n = 60) aged between 5 and 7 years old and taken home to parent/carer. As a result, 49 children agreed to take part in the study. Data collection for the study took place between November-December, 2017.

121 Data Collection and Procedures

All the participants were invited to take part in a standardised activity protocol and to be video-recorded during school recess. Data collection took place in the school gymnasium and playground to mimic free-living conditions and increase the ecological validity of the study protocol. Children's stature (The Leicester Height Measure, Child Growth Foundation, Leicester, United Kingdom), sitting stature and waist circumference to the nearest 0.1cm together with mass to the nearest 0.1kg (model 760, Seca, Hamburg, Germany) were measured using standard procedures (22). All measurements were taken twice, with a third measurement taken if the first two differed by more than >1%. Body mass index (BMI) was calculated from stature and mass. Children self-reported their dominant hand and additionally they were asked to write their name on a paper so researchers could double check hand dominance.

132 *Activity monitors*

Participants were fitted with an ActiGraph GT9X Link on both wrists and on the right hip, and wore the devices throughout the data collection session. The GT9X was set to record at 100Hz and measured acceleration in a range of $\pm 8g$ on x, y and z axes. Data were downloaded in 1s epochs.

137 *Direct observation*

138 Children's SB and PA were assessed using direct observation during the standardised 139 activity protocol and during recess. Direct observation was chosen as the criterion reference 140 for the classification of SB and PA levels as it is considered the most appropriate method to 141 assess rapid changes in physical activity behaviours, typical of this age group, it does not 142 involve equipment that might impair children's normal movements (17) and has been used for 143 calibration purposes in previous studies (23, 24).

144

145 *Calibration Protocol*

The activity protocol lasted around 60 minutes in total, took place in the school hall during usual lesson time, and involved three participants at a time, rotating between 10 different tasks (Table 1). The selection of the tasks was informed by previous calibration studies in this age group, by observing children's typical recess play activities, and through consulting primary school teachers. Tasks were selected to encompass each activity intensity (SB, LPA, 151 MPA and VPA) and were designed to simulate children's free-living PA and SB as accurately as possible. Four SB (Lying while watching TV, sitting while colouring, sitting and play with 152 a tablet and playing with LEGO), one LPA (passive standing), two MPA (walking briskly 153 together, throwing and catching) and three VPA (running, obstacle course run and hopping) 154 activities were included in the protocol. The intensity of each activity in the protocol was 155 classified using METs as reported in the youth compendium of physical activities (25). The 156 most widely accepted intensity thresholds were used to classify the activities: SB (≤ 1.5 METs), 157 LPA (≥1.5–<3METs), MPA (≥3–<6 METs), VPA (≥ 6 METs) (26). 158

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- 160

[TABLE 1 ABOUT HERE]

The activities were ordered into three different activity protocols and participants were randomised to one of the protocols. The three protocols were designed to allow three children to complete the protocol simultaneously. Children had 2 minutes rest after MPA and VPA tasks, while they were asked whether they needed more rest before starting each activity. Researchers independently conducted live direct observations of children through the protocol, which involved continuously instructing and supervising children to ensure they were 'on task', and recording the start time and end times of each activity.

168 *Recess observation*

Recess was included in the study protocol to capture children's behaviours during freeliving conditions. Children were asked to participate in school recess as normal whilst wearing the devices. Each researcher video-recorded one child for a period of 10 minutes during either morning or lunchtime recess. Based on previous studies measuring activity levels during recess and previous observations of children's recess in the school involved, we expected children to spend the highest proportion of recess in LPA and a progressively lower amount of time in MPA, VPA respectively (27). Behaviours during recess were assessed and classified on a 176 second-by-second basis (in order to match accelerometery 1s epochs) using the Youth compendium of physical activities (25). Before proceeding with the video analysis, the research 177 team analysed three randomly selected video-recordings jointly in a single group session where 178 behaviour classification was discussed until unanimous consensus was reached. Subsequently, 179 one researcher classified children's recess behaviours second-by-second based on the activities 180 and METs reported in the Youth compendium of physical activities (SB: ≤1.5METs, LPA: 181 >1.5&<3METs, MPA: \geq 3&<6 METs, or VPA: \geq 6 METs) (25). Uncertainties with the 182 classification of children's behaviours that emerged during analysis were discussed and 183 184 resolved with the research team by consensus.

185 *Data analysis*

ActiGraph accelerations were downloaded and converted to .csv format data using 186 187 Actilife software (ActiLife v6.13.3). Subsequently, the package GGIR version 1.11-0 from R software version 3.2.5 (R Foundation, www.r-project.org) was used to process raw data and 188 calculate average ENMO accelerations for each 1 second epoch. As a result, csv documents 189 presenting ENMO and related timestamps were produced. Acceleration data were then paired 190 with SB and PA observation data. The first and last 15 seconds of each task in the activity 191 protocol were deleted to account for possible start and end time imprecision, transition time 192 delays, and irregular movement patterns, as well as to control for learning effect and fatigue. 193 Only data from participants that completed both the standardised protocol and observation of 194 195 recess were included in the final analysis. The final sample of participants was randomly divided into a cut-point generation (22 participants, n = 11 girls) and a cross-validation (10 196 participants, n = 6 girls) group for analysis. Shapiro Wilk test was performed to assess 197 198 distribution normality of decimal age, height, weight, BMI both in participants included and excluded from the study. Subsequently, either independent samples t-test or Mann-Whitney 199 test were performed to assess differences in decimal age, height, weight and BMI between 200

participants in the two groups based on normality distribution test. Differences in the
distribution of males and females between participants included and excluded was assessed
using Chi-square test.

In this study we proposed a novel approach to cut-point calculation divided in 3 phases comprising 1) initial ROC analysis, 2) the use of equivalence testing to identify the likely optimum cut-points at the group level and 3) cross validation of the cut-points.

Phase 1. During the first phase cut-points were calculated using ROC curve analysis in
the cut-point generation group. R package pROC was used to perform ROC and calculate SB,
MVPA and VPA cut-points.

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[TABLE 2 ABOUT HERE]

Consistent with previous studies, ROC analysis was initially performed including all 211 212 the SB and PA levels (i.e. all recorded data across all activities). In contrast to previous research, and to reduce bias associated with unequal distributions of PA behaviours (28), ROC 213 analysis was performed including pairs of activity levels, for example: SB versus LPA, MPA 214 versus VPA (Table 2). To evaluate the effect of passive standing on the diagnostic accuracy 215 of the cut-points, the acceleration signals collected during standing while watching TV were 216 excluded from some of the conditions ROC analysis (Table 2). The Youden index and Distance 217 method (selecting the point in the ROC curve that is closer to the left corner of the ROC curves 218 plot) were used to calculate cut-points (29). The Area Under the ROC curve (AUC) and the 219 220 related confidence interval (ciAUC) were calculated as a measure of a test's ability to discriminate between different conditions. Sensitivity and specificity were calculated. 221 Agreement between the criterion method (direct observation) and accelerometer estimates 222 223 generated using the cut-points was assessed using % of agreement (%Ag) and Cohen's Kappa (CK). CK values were considered poor when lower than 0.00, slight when between 0.00 and 224 0.20, fair when between 0.21 and 0.40, moderate when between 0.41 and 0.60, substantial when 225

226 between 0.61 and 0.80 and almost perfect when between 0.81 and 1.00 (30). Lastly, equivalency analysis was used to assess the group-level equivalence between the observation 227 and cut-point derived SB and PA estimates (31). Equivalency analysis compares an 228 229 equivalence region derived from a criterion reference (e.g. observation) to the confidence interval for the difference in means between the criterion reference and a different method (e.g. 230 accelerometery). The equivalence region is centred on the mean derived from the criterion 231 232 reference while the confidence interval is centred on the mean obtained from the method to compare. Non-equivalence is rejected at the level α if 100(1-2 α)% confidence interval for the 233 234 difference in means lies entirely within the equivalence region. Based on previous research using equivalency testing to compare PA assessment methods, we used an equivalence region 235 of $\pm 10\%$ the mean of the time spend in SB or PA activities assessed using the criterion method 236 237 (observation) (32). Subsequently, we calculated the 90% confidence interval (as α was set at 0.05) for the difference in means between observed and cut-points derived time spent in SB 238 and PA activities. Cut-point derived estimates were considered equivalent if the 90% 239 confidence interval of the difference in means fell within the $\pm 10\%$ equivalence region. 240

Phase 2. Time spent in SB and PA levels derived from observation and ROC analysis generated cut-points were compared using equivalency. Subsequently, the most accurate cutpoints were increased or decreased by 1mg progressively until cut-points providing the optimum estimates at the group-level (based on equivalency analysis) of SB, MVPA and VPA respectively were identified. Sensitivity, specificity, %Ag, and CK were re-examined for the revised cut-points and relative Bland Altman plots were produced (33).

Phase 3. In the third phase, the revised cut-points developed in phase 2 were applied to
the cross-validation group. In this phase agreement and accuracy were calculated for SB, LPA,
MPA, MVPA and VPA. Sensitivity, specificity, %Ag, CK were calculated and equivalency

analysis was performed. Additionally, Mean absolute percent error (MAPE) was calculated asan individual-level measure of error and relative Bland Altman plots were produced.

252

253 **Results**

Forty-nine children (45% male;) agreed to take part in the study. Seventeen children 254 did not complete the recess observation due to poor weather (heavy rain, icy conditions) and 255 time constraints (data collection was restricted to December 2017). Thirty-two children (47% 256 male;) completed all the assessments and were therefore included in the final analysis. The 257 258 children who completed all the assessment included 12 children aged 5 years, 12 children aged 6 years and 8 children aged 7 years. Participant characteristics can be found in Table 3. No 259 significant differences (p>0.05) were found between participants included and excluded from 260 261 the analysis in terms of gender, decimal age, height, weight and BMI.

262

[TABLE 3 ABOUT HERE]

Children were video recorded during recess for an average of 7 minutes and 17 seconds (range: 3 minutes and 35 seconds to 10 minutes and 11 seconds). Table 4 presents mean ENMO, standard deviation and number of observations for each activity children engaged in during the standardised activity protocol and recess.

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[TABLE 4 ABOUT HERE]

Phase 1: Cut-points calculated using the Youden and Distance methods are presented in Supplementary material 1 (see Supplementary Tables 1, 4 and 5). Most of the AUC were higher than 0.7 apart from "SB=1 and LPA=0" in the dominant wrist and hip placement with AUC equal to 0.611 and 0.689, respectively. The majority of cut-points presented higher sensitivity than specificity. Sensitivity ranged from 65.3% to 99.1% while specificity ranged from 61.8% to 96.5%. In terms of agreement, %Ag ranged from 71.5% to 95% while CK ranged from 0.43 to 0.82 representing moderate to substantial agreement. Cut-points that included all the SB and PA levels in the ROC analysis generally presented higher AUC, higher sensitivity and lower specificity compared to the cut-points developed using pairs of activity levels. Moreover, the cut-points that included all SB and PA levels generally presented better agreement with observation for SB and lower agreement with observation for MVPA and VPA compared to cut-points developed using pairs of activity levels. Furthermore, excluding standing while watching TV from the ROC analysis resulted in an increase in AUC for SB and a decrease in the AUC for MPA and VPA ROC curves.

Based on the equivalency analysis (Figures 1-3) the cut-points developed using paired activity levels provided a better group-level estimate of time spent in SB, MVPA and VPA compared to cut-points developed using all the SB and PA levels (see CK and %Ag reported in Supplementary material 1: Supplementary Tables 1, 4 and 5). In general, Distance cut-points provided better estimates of SB, MVPA and VPA compared to Youden cut-points.

Phase 2: Results from phase 2 can be found in the Supplementary material 1 287 (Supplementary Tables 1-5). The cut-points providing the most comparable estimates of SB, 288 MVPA and VPA were identified using equivalency testing (See Figures 1-3). Sensitivity, 289 specificity, %Ag and CK observed in phase 2 cut-points were either similar or higher compared 290 291 to the those observed in phase 1 meaning that cut-points developed in phase 2 obtained higher agreement with the criterion reference for SB and PA. SB cut-points demonstrated lower % Ag 292 293 and CK compared to the MVPA and VPA cut-points. Based on equivalency analysis, the 294 amount of time spent in SB, MVPA and VPA calculated using phase 2 cut-points was equivalent on average at the group level to the observed values with the exception of the SB 295 hip accelerometer cut-point. LPA and MPA displayed lower agreement with the observed 296 297 values in comparison to other PA levels. Wider limits of agreement where observed in Bland Altman plots for hip SB and LPA cut-points compared to wrist cut-points (see Supplementary 298 material 2: Supplementary Figures 1-6). Furthermore, a linear relation between bias and 299

average of the differences was observed in Bland Altman plots of SB (Supplementary material
2: Supplementary Figures 1-3) as children engaged in approximatively the same amount of SB
(23min).

303 [FIGURE 1 - 2 - 3 ABOUT HERE]

304 *Phase 3*: The final cut-points developed in phase 2 were applied to the cross-validation305 group and the results are presented in Table 5.

306 [TABLE 5 ABOUT HERE]

Consistent with phase 2, SB cut-points demonstrated lower %Ag and CK compared to 307 308 MVPA and VPA cut-points. LPA and MPA displayed lower agreement with the observed values in comparison to other PA levels with sensitivity between 27.4%-39.8%, specificity 309 between 78.5%- 94.3%, %Ag between 67.5%- 87.7% and CK between 0.06-0.36. Based on 310 311 the equivalency analysis, estimates were equivalent on average at the group level for SB, and MVPA for non-dominant wrist cut-points, and for SB for the dominant wrist cut-points. No 312 estimates were considered equivalent for the hip placement. Non-dominant wrist placement 313 showed slightly higher CK and %Ag together with lower MAPE and better results in 314 equivalency analysis compared to hip placement in SB and LPA classification (Figure 4). 315 Similarly, non-dominant wrist placement showed higher CK and %Ag compared to dominant 316 wrist placement in SB and LPA classification. Wider limits of agreement were observed in 317 Bland Altman plots for hip SB and LPA cut-points (Supplementary material 2: Supplementary 318 319 Figures 16-21) compared to wrist cut-points confirming results from equivalency analysis and MAPE. In line with what observed in phase 2, a linear relation between bias and average of the 320 differences was observed in Bland Altman plots of SB (Supplementary material 2: 321 322 Supplementary Figures 16-18).

323 [FIGURE 4 ABOUT HERE]

324

325 Discussion

This study developed raw acceleration SB and PA cut-points in 5-7 year old children 326 for wrist and hip worn accelerometers. SB, MPA, MVPA and VPA cut-points demonstrated 327 328 adequate levels of agreement (i.e. fair to substantial CK agreement, %Ag \ge 73%) and error (MAPE $\leq 21.6\%$) with the criterion reference for all Accelerometer placements. LPA 329 measurement presented lower agreement with the criterion method compared to SB, MPA, 330 331 MVPA and VPA, in line with findings observed in previous studies (34) with higher levels of error reported in hip placement (MAPE = 51.9%) compared to non-dominant (MAPE = 19.6%) 332 333 and dominant placement (MAPE = 18.6%). However, the %Ag observed in this study in LPA classification was higher than the one observed in previous literature (34) suggesting that the 334 cut-points are adequate for the use in the field. Non-dominant wrist cut-points performed 335 336 slightly better than other placements in assessing SB and LPA behaviours presenting higher levels of %Ag and CK compared to both dominant wrist and hip placement together with lower 337 levels of MAPE, better agreement in equivalency analysis and smaller confidence interval in 338 Bland Altman plots compared to hip placements for SB and LPA. Not surprisingly, SB cut-339 points presented lower agreement with the criterion reference compared to MVPA and VPA 340 cut-points confirming the known limitations of accelerometers when aiming to distinguish SB 341 from passive standing LPA (15). This study also demonstrated that combining equivalency 342 analysis with ROC analysis could lead to more accurate cut-points than the ones derived from 343 344 ROC analysis alone, based on the higher levels of agreement observed in Phase 2 compared to Phase 1 of the statistical analysis we reported. 345

346 SB cut-points were higher at the wrist than hip placement (36mg, 39mg and 20mg for 347 non-dominant wrist, dominant wrist and hip placement respectively), in line with the majority 348 of cut-points developed in previous literature (18). However, the opposite was reported by 349 Hildebrand et al. (15) who created SB cut-points for ActiGraph accelerometers using ENMO in a similar older age group (7-11 years old). Hildebrand et al. (15) obtained higher cut-points for the hip placement compared to wrist placement (63.3mg and 35.6mg for hip and nondominant wrist placement, respectively). Possible reasons behind this inconsistency in hip placement cut-points could be that Hildebrand et al. (15) utilised different activities in their protocol, used the Youden method alone in the ROC analysis to identify cut-points, and involved a different criterion reference (i.e. activPAL).

356 Interestingly, higher sensitivity than specificity values were observed in Hildebrand et al. (15) and in our study. Hildebrand et al. (15) argued that the lower levels of specificity might 357 358 be due to the inclusion of standing as LPA in the study protocol. Passive standing might lead to the absence of registered accelerations or low accelerations similar to SB activities. Despite 359 being classified as LPA based on energy expenditure and/or the posture, standing watching TV 360 361 does not necessarily involve movement and therefore could be classified as passive standing (35). Previous research has demonstrated the limitations of accelerometers in distinguishing 362 stationary behaviours such as passive standing from SB (20, 36). Another limitation of SB 363 assessment using cut-points in is the lack of consideration of posture that is a key aspect of SB 364 identification (37). This is confirmed by the results of our study where the mean acceleration 365 during passive standing (Table 4) was below the SB cut-points. 366

SB raw acceleration cut-points have been developed by Schaefer et al. (34) and Duncan 367 et al. (38) in GENEActiv devices for children aged between 5-7, though, rather than using 368 369 ENMO these studies utilised different metrics to represent acceleration signals. SB cut-point presented in both Schaefer et al. (34) and Duncan et al. (38) studies were higher than SB cut-370 points developed in this study (36mg, 39mg, 20mg) with values of 190mg and 75mg (converted 371 372 from time to independent unit mg) respectively. This is in line with previous studies where higher accelerations were observed in GENEActiv compared to ActiGraph when measuring 373 the same participants simultaneously (39). However, key reasons for the disparity in cut-points 374

is likely due to the different metrics that have been used to represent the acceleration meaningcut-points are not directly comparable (18).

Hildebrand et al. developed MVPA and VPA cut-points for ActiGraph using ENMO 377 in 7-11 year old children. Their reported cut-points were higher for both wrist (MVPA: 378 201.4mg, VPA: 707.0mg) and hip (MVPA: 142.6mg, VPA: 464.6mg) placements compared 379 to the ones in our study (MVPA: 189mg for non-dominant wrist, 181mg for dominant wrist 380 and 95mg for hip; VPA: 536mg for non-dominant wrist, 534mg for dominant wrist and 325mg 381 for hip) (Table 5). There are several potential reasons for the differences between the 382 383 Hildebrand cut-points and the ones reported in the present study. For example, the difference in age range between the participants involved, the use of indirect calorimetry as criterion 384 reference rather than observation, using linear regression for cut-points identification and the 385 386 use of different activities within the study protocol (14). Van Loo et al. (40) assessed the accuracy of three sets of MVPA and VPA raw accelerometers cut-points developed by 387 Hildebrand et al. (15) Philips et al. (41) and Schaefer et al. (34) for GENEActiv wrist mounted 388 389 devices in 5-8 year old children and found that these cut-points led to considerable misclassification of PA levels. Interestingly, none of the cut-points examined by van Loo et al. 390 (40) were originally developed from a sample of 5-8 years old children (15, 34, 41) and 391 therefore it is possible that they were not adequate for the classification of MPA, MVPA and 392 VPA in that age group. 393

When considering previous studies that examined raw acceleration cut-points in 5-7 year old children, only Schaefer et al. (34), Hildebrand et al. (14) and Van Loo et al. (40) reported %Ag. Schaefer et al. (34) reported slightly higher %Ag for the SB cut-point (83.3%) but lower %Ag for LPA (29.4%), MPA (41%) and VPA (88.7%) compared to our study (%Ag in this study: SB between 73% and 78.5%, LPA between 67.5% and 62.5%, MPA between 88.7% and 88.2%, VPA between 92 and 93.8%). Similarly, Hildebrand et al. (14) and Van Loo

et al. (40) obtained lower % Ag for MPA and VPA (% Ag for Hildebrand et. (14): MPA between 400 33% and 55%, VPA between 68% and 80%; %Ag for Val Loo et al. (40): MPA between 45.4% 401 and 52%, VPA between 70% and 93.6%). In this study according to Cohen's Kappa values, 402 403 LPA estimates demonstrated slight agreement, while MPA estimates showed fair agreement, and SB, MVPA and VPA moderate to substantial agreement. Given that no previous calibration 404 studies in this age group have reported CK, we suggest that future studies should include this 405 406 measure of reliability to account for chance agreements. Overall, the %Ag reported in this study is higher than those observed in previous studies applying raw acceleration cut-points in 5-7-407 408 year-old children, demonstrating that the cut-points proposed in this study could lead to improved accuracy in PA assessment. 409

A major strength of this calibration study was its high ecological validity as the protocol 410 411 included direct observation of children's SB and PA during recess within the school playground 412 and during a standardised protocol of activities performed in their physical education hall. Additionally, this is the first accelerometer calibration study in this age group to consider 413 different methods of cut-point calculation, including: i) exploring the use of paired activity 414 levels in ROC curve analysis, ii) examining the Youden and distance methods for cut-point 415 development, and iii) using equivalency methods to identify and refine cut-points. Further 416 strengths are the use of the ENMO metric, emerging as the most frequently used metric to 417 process raw acceleration and generate thresholds for multiple accelerometer placements (42). 418

Despite the advantages of using direct observation as criterion reference for SB and PA assessment exposed in our methods section, we acknowledge that direct observation is not the gold standard for the measurements of energy expenditure and presents a level of subjectivity. Furthermore, because of time constraints and participants' availability, it was not possible for the all the initial 49 participants to complete the study protocol and to obtain a balanced number of children within each age group involved in the study (12 children aged 5 years, 12 children 425 aged 6 years, and 8 children aged 7 years). We recognise that the limited number of children 426 in the cut-point generation group together with the use of statistical analysis methods maximizing accuracy might lead to over fitting related problems. For future calibration studies, 427 428 we suggest involving an equal number of participants in each age group to guarantee that each age is equally represented in the sample, together with a bigger sample size to guarantee a 429 better representation of the population. In line with previous research, we encountered 430 difficulties in the selection of standardised LPA activities for the testing protocol. Similar to 431 previous studies (15, 40, 43), we classified slow walking and standing as LPA. Given that 432 433 passive standing might lead to misclassification of SB and LPA, other activities that are representative of 5-7 years old children free-living LPA should be identified in the future. 434 Moreover, future studies should examine methods to integrate postural aspects to the 435 436 measurement to account for accelerometers limitations in classifying sedentary behaviours.

437

438 Conclusions

SB, LPA, MPA, MVPA and VPA cut-points demonstrated adequate accuracy in all 439 accelerometer placements. Non-dominant accelerometer placement presented slightly better 440 agreement with the criterion reference compared to the dominant wrist and hip placements for 441 SB and LPA. However, no other differences were highlighted between the accelerometer 442 placement. These findings can be used to inform the decisions made by researchers in relation 443 444 to the assessment of young children's PA and SB. Furthermore, the study protocol, methods and analysis can inform the development of more rigorous calibration studies and subsequent 445 analyses to determine cut-points in the future. In view of our results, we suggest that cut-points 446 447 developed using Youden method involving all SB and PA levels in ROC analysis can lead to large misclassification of SB and PA levels. Future researchers should include paired activity 448

449	levels analysis together with distance method in ROC analysis in combination with equivalency
450	analysis and Cohen's Kappa statistic to select the most accurate SB and PA cut-points.
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458	contributed in the data collection.
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460	Conflict of interest
461	No conflict of interest was reported between authors and other people involved in the study.
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597	Exerc. 2017;49(4):813.							
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601	Table	1: Standardised activit	y protocol					
	Sedentary behaviours							
	Lying	g while watching TV	Lie comfortably on a mat while watching an age appropriate television programme or movie for 10 minutes.					
	Sittir	ng while colouring	Colouring exercise while sitting at a table for 5 minutes.					
	Sittir	ng playing with a tablet	Play games on a tablet while sitting on a chair for 5 minutes.					
	Playing with LEGO Sit or lie on the floor while playing with Lego for 5 minutes.							
	Ligh	t physical activity						
	Stand	ding while watching TV	Stand and watch a video for 5 minutes.					
	Mod	erate physical activity						

Moderate physical activity	
Walking briskly self-paced	Walk briskly for 2 minutes, at a self-selected pace around a designated track or circuit. A researcher walked with the child encouraging him/her to maintain the pace.
Throwing and catching	Child and researcher passed the ball to each other continuously for 2 minutes.
Vigorous physical activity	
Dermalar	
Running	Run for 2 minutes, at a self-selected pace around a designated track or circuit.
Obstacle course	
C	track or circuit. Run for 2 minutes on a course around cones. This course was designed to mimic typical run/chase type activities and

Table 2. Dichotomization of the data for the ROC analysis

	Sedentary					
	"1"	" 0 "				
	SB	LPA, MPA, VPA.				
	SB	LPA excluding standing while watching TV, MPA, VPA				
	SB	LPA				
	SB	LPA excluding standing while watching TV				
	Moderate physical activity					
	"1"	" 0 "				
	MPA,VPA	SB, LPA				
	MPA,VPA	SB, LPA excluding standing watching TV				
	MPA	LPA				
	MPA	LPA excluding standing watching TV				
	Vigorous physical activity					
	"1"	" 0 "				
	VPA	SB, LPA, MPA.				
	VPA	SB, LPA excluding standing watching TV, MPA				
	VPA	MPA				
613	Scored "1" when the condition	n is present; Scored "0" when the condition is absent; SB:				
614	Sedentary behaviours; LPA: Li	ght physical activity; MPA: Moderate physical activity; VPA:				
615	Vigorous physical activity.					
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Table 3. Participants' descriptive data 627

Initial group (n=49)

Height (cm)

Weight (Kg)

BMI (Kg/m²)

Males (n=2	22)	Females (n=27)			
Mean	SD	Mean	SD		
6.5	0.8	6.5	0.7		
120.2	6.7	120.4	9.0		
23.6	3.9	24.4	6.1		
16.3	1.8	16.6	2.1		
	Mean 6.5 120.2 23.6	6.50.8120.26.723.63.9	MeanSDMean6.50.86.5120.26.7120.423.63.924.4		

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SD

0.7

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Final group (n=32)					
	Males (n=	15)	Females (n=17)		
	Mean	SD	Mean	S	
Decimal age (years)	6.4	0.8	6.4	0	

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644Table 4. Accelerations observed in each SB and PA level recorded

				Non-		Domin	ant	Hip	
Intensity (MET)	Standardised Protocol	MET	Obs (s)	Mean (mg)	SD (mg)	Mean (mg)	SD (mg)	Mean (mg)	SD (mg)
Sedentary	Lying while watching TV	1.2	18155	17	37	15	37	12	14
	Sitting while colouring	1.6	8640	20	47	37	65	11	13
	Sitting and playing with a tablet	1.4	8640	11	21	23	28	9	12
	Playing with LEGO	1.5	8640	52	48	51	47	11	12
Light	Standing	1.7	8640	20	39	12	27	9	13
Moderate	Walking briskly self- paced	4.6	2880	294	289	255	271	178	100
	Throw and catch	4.9	2790	444	370	432	374	83	88
Vigorous	Running	7.8	2865	1071	581	1115	601	607	179
	Obstacle course	7.2	2880	744	424	719	396	446	165
	Hopping	6.3	2563	844	552	762	491	452	241
	Recess								
Sedentary	Sitting down	1.4	51	64	64	67	80	18	27
Light	Standing	1.7	3007	103	165	117	210	45	88
	Walk slow	2.5	6164	204	249	207	266	120	128
Moderate	Walk brisk	4.6	665	528	397	473	398	336	196
	Jog slow	5.5	1364	652	459	644	537	434	259
	Dancing	3.6	13	654	557	347	340	162	126
	Ball games	6.0	23	773	337	652	379	379	189

	Jumping-jack	5.9	107	931	463	1081	449	281	247
Vigorous	Jog fast	6.8	1178	1103	632	1032	688	599	290
	Running	7.8	510	1772	894	1766	999	808	254
	Hopping	6.3	437	883	537	782	575	528	259
	Jump rope	6.9	577	801	390	1140	456	649	241
	Ball games	6.1	75	1663	696	1347	633	604	204

645 646 647 Obs: Number of observation of each behaviours where each observation corresponds to 1 second spent in the

activity observed. **MET:** Metabolic equivalent (1 MET equals the oxygen uptake of $3.5 \text{mL} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}$)

							an derived	Equivalency analysis lerived mean and onfidence interval	
	Cut-point (mg)	Sn (%)	Sp (%)	CK (a.u)	%Ag (%)	MAPE (%)	Obs (min)	Cut-point (min)	
Non-									
dominan wrist	IT								
SB	<36	79.8	56.8	0.57	78.5	9.3	23.0±2.3	22.8±1.4	
LPA	≥36&<189	38.4	81.9	0.20	72.5	19.6	9.1±0.9	9.5±1.2	
MPA	≥189&<536	39.0	93.7	0.34	87.7	19.0	4.7±0.5	4.2±0.6	
MVPA	≥189	82.6	78.0	0.78	92.0	9.0	10.2±1.0	10±0.8	
VPA	≥536	75.1	68.7	0.69	92.7	12.9	5.5 ± 0.6	5.9 ± 0.5	
Dominar	Dominant								
wrist									
SB	<39	75.4	70.2	0.46	73.0	10.1	23.0±2.3	23.1±1.7	
LPA	≥39&<181	27.4	78.4	0.06	67.5	18.7	9.1±0.9	9.6 ± 1.2	
MPA	≥181&<534	39.8	93.5	0.35	87.7	14.4	4.7 ± 0.5	4.3±0.5	
MVPA	≥181	79.1	76.0	0.76	91.4	13.5	$10.2{\pm}1.0$	9.5±1.0	
VPA	≥534	67.6	95.6	0.64	92.0	16.2	5.5 ± 0.6	5.3±0.7	
Нір									
SB	<20	78.0	50.1	0.50	75.3	21.2	23.0 ± 2.3	23.3±3.1	
LPA	≥20&<95	30.0	80.2	0.10	69.4	51.9	9.1±0.9	9.3±3.0	
MPA	≥95&<325	39.1	94.3	0.36	88.2	21.6	4.7±0.5	4±0.7	
MVPA	≥95	79.3	75.6	0.76	91.2	13.2	10.2±1.0	9.7±1.0	
VPA	≥325	78.2	96.1	0.73	93.8	11.3	5.5±0.6	5.7±0.4	

648 Table 5. Cut-points performance in cross-validation group

649**SB:** Sedentary behaviours; **LPA:** Light physical activity; **MPA:** Moderate physical activity; **MVPA:** moderate to650vigorous physical activity; **VPA:** Vigorous physical activity; **Sn:** Sensitivity; **Sp:** Specificity; **CK:** Cohen's651Kappa; **%Ag:** Percentage of agreement. **MAPE:** mean absolute percent error; **a.u.:** Arbitrary units; **Obs:**652Concerns the mean time spent in SB and PA levels obtained by observation $\pm 10\%$ of the mean time spent in a653specific activity level derived from observation; **Cut-point:** Concerns the mean of the cut-points derived SB and654PA levels and the related 90% confidence interval of the difference between observed and cut-point derived655minutes spent in a specific activity level.

656 Figure 1. Non-dominant wrist equivalency analysis in Cut-point generation group (Phase

1-2)

658 [FIGURE 1 ABOUT HERE]

- *: the cut-points marked with a * were calculated using ROC analysis Youden method.
- 660 #: the cut-points marked with a # were calculated using ROC analysis Distance method.
- **Phase 2:** the cut points in Phase 2 was calculated using equivalency analysis method.
- 662 Solid line: The solid line concerns the 90% confidence interval of the difference between observed and cut-
- 663 point derived minutes spent in a specific activity level. The confidence interval is centred on the mean of the
- 664 cut-point derived time estimate of the activity level taken into consideration (i.e. SB, MVPA, VPA).
- **Dashed line:** The dashed line concerns the $\pm 10\%$ interval of the mean time estimate of a specific activity level
- 666 calculated using observation. The $\pm 10\%$ interval is centred on the mean of the observation derived time estimate 667 of the activity level taken into consideration (i.e. SB, MVPA, VPA).

Figure 2. Dominant wrist equivalency analysis in Cut-point generation group (Phase 1-2)

688 [FIGURE 2 ABOUT HERE]

*: the cut-points marked with a * were calculated using ROC analysis Youden method.

690 #: the cut-points marked with a # were calculated using ROC analysis Distance method.

691 **Phase 2:** the cut points in Phase 2 was calculated using equivalency analysis method.

692 Solid line: The solid line concerns the 90% confidence interval of the difference between observed and cut-

693 point derived minutes spent in a specific activity level. The confidence interval is centred on the mean of the

694 cut-point derived time estimate of the activity level taken into consideration (i.e. SB, MVPA, VPA).

695 Dashed line: The dashed line concerns the $\pm 10\%$ interval of the mean time estimate of a specific activity level

696 calculated using observation. The $\pm 10\%$ interval is centred on the mean of the observation derived time estimate

697 of the activity level taken into consideration (i.e. SB, MVPA, VPA).

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718 Figure 3. Hip equivalency analysis in Cut-point generation group (Phase 1-2)

719 [FIGURE 3 ABOUT HERE]

*: the cut-points marked with a * were calculated using ROC analysis Youden method. #: the cut-points marked with a # were calculated using ROC analysis Distance method. Phase 2: the cut points in Phase 2 was calculated using equivalency analysis method. Solid line: The solid line concerns the 90% confidence interval of the difference between observed and cut-point derived minutes spent in a specific activity level. The confidence interval is centred on the mean of the cut-point derived time estimate of the activity level taken into consideration (i.e. SB, MVPA, VPA). **Dashed line:** The dashed line concerns the $\pm 10\%$ interval of the mean time estimate of a specific activity level calculated using observation. The $\pm 10\%$ interval is centred on the mean of the observation derived time estimate of the activity level taken into consideration (i.e. SB, MVPA, VPA).

749 Figure 4. Standard confidence interval test in cross validation group (Phase 3)

750 [FIGURE 4 ABOUT HERE]

- 751 SB: Sedentary behaviours; LPA: Light physical activity; MPA: Moderate physical activity; MVPA: moderate to vigorous physical activity; VPA: Vigorous physical activity.
- 753 Solid line: The solid line concerns the 90% confidence interval of the difference between observed and cut-
- point derived minutes spent in a specific activity level. The confidence interval is centred on the mean of the
- cut-point derived time estimate of the activity level taken into consideration (i.e. SB, LPA, MPA, MVPA,
- 756 VPA).
- **Dashed line:** The dashed line concerns the $\pm 10\%$ interval of the mean time estimate of a specific activity level
- calculated using observation. The $\pm 10\%$ interval is centred on the mean of the observation derived time estimate
- of the activity level taken into consideration (i.e. SB, LPA, MPA, MVPA, VPA).
- 760