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Validity of hip-mounted uniaxial accelerometry with heart-rate monitoring vs. triaxial accelerometer in the assessment of free-living energy expenditure in young children: the IDEFICS Validation Study

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Ojiambo R, Konstabel K, Veidebaum T, Reilly J, Verbestel V, Huybrechts I, Sioen I, Casajús JA, Moreno LA, Vicente-Rodriguez G, Bammann K, Tublic BM, Marild S, Westerterp K, Pitsiladis Y, IDEFICS Consortium. Validity of hip-mounted uniaxial accelerometer with heart-rate monitoring vs. triaxial accelerometer in the assessment of free-living energy expenditure in young children: the IDEFICS Validation Study. J Appl Physiol 113: 1530–1536, 2012. First published September 20, 2012; doi:10.1152/japplphysiol.01290.2011.—One of the aims of the Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants (IDFICS) validation study is to validate field measures of physical activity (PA) and energy expenditure (EE) in young children. This study compared the validity of uniaxial accelerometer with heart-rate (HR) monitoring vs. triaxial accelerometer against doubly labeled water (DLW) criterion method for assessment of free-living EE in young children. Forty-nine European children (25 female, 24 male) aged 4–10 yr (mean age: 6.9 ± 1.5 yr) were assessed by uniaxial ActiTrainer with HR, uniaxial 3DNX, and triaxial 3DNX accelerometer. Total energy expenditure (TEE) was estimated using DLW over a 1-wk period. The longitudinal axis of both devices and triaxial 3DNX counts per minute (CPM) were significantly (P < 0.05) associated with physical activity level (PAL); r = 0.51 ActiTrainer, r = 0.49 uniaxial-3DNX, and r = 0.42 triaxial 3DNX). Eight-six percent of the variance in TEE could be predicted by a model combining body mass (partial r² = 71%; P < 0.05), CPM-ActiTrainer (partial r² = 11%; P < 0.05), and difference between HR at moderate and sedentary activities (ModHR – SedHR) (partial r² = 4%; P < 0.05). The SE of TEE estimate for ActiTrainer and 3DNX models ranged from 0.44 to 0.74 MJ/day or ~7–11% of the average TEE. The SE of activity-induced energy expenditure (AEE) model estimates ranged from 0.38 to 0.57 MJ/day or 24–26% of the average AEE. It is concluded that the comparative validity of hip-mounted uniaxial and triaxial accelerometers for assessing PA and EE is similar.

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Physical activity (PA) is a complex behavior that varies with age, gender, season, weekday, and time of the day and is also influenced by biological, sociological, psychological, and environmental factors (1). A decline in PA has been identified as an important contributory factor in childhood obesity (24) but is difficult to quantify precisely, as few studies utilize the same methods of assessment and limited objective data exist (1, 24). PA is generally considered to be a central factor in the etiology, prevention, and treatment of childhood obesity (37), and thus the quantification of energy expenditure (EE) and daily PA has gained considerable interest (9). However, accurate assessment of PA and sedentary behavior, especially in children remains a significant challenge (1). Consequently, validated techniques of estimating habitual PA are needed to study the relationship between free-living PA and obesity (37). These methods should be suitable to measure PA over periods long enough to be representative for normal daily life, with minimal discomfort to the subjects, and applicable to large study populations (37).

The ability to accurately track EE using objective methods is crucial (11), especially in studies that aim to track the trends in EE over time. Doubly labeled water (DLW) is the only technique available to accurately measure total energy expenditure (TEE) over prolonged periods in daily life (5). When this technique is combined with a measure of basal metabolic rate (BMR), activity-induced energy expenditure (AEE) can be calculated; i.e., AEE = 0.9 × TEE − BMR or physical activity level (PAL) = TEE/BMR (19). However, since DLW is markedly expensive and requires appropriate laboratory equipment for sample analysis, it is infrequently used in large-population studies to assess TEE and related correlates. Thus
other methods such as uniaxial accelerometry have been proposed to assess PA and to estimate TEE (5). Accelerometers provide a means by which researchers can examine the intensity, frequency, and duration of PA bouts that individuals are performing over extensive periods of time (10). By validating an accelerometer against DLW-derived EE, prediction models can be developed to predict AEE, TEE, or PAL from accelerometer counts and other physical characteristics, such as age, sex, height, and body mass (BM; Ref. 19). Thus improvement of the accuracy of accelerometers in predicting TEE, AEE, and PAL has been the focus of several studies in adults (4, 9, 20). However, there has been less examination of the validity of accelerometers against DLW in very young children.

Uniaxial accelerometers measure accelerations in one plane (usually longitudinal axis of the body), whereas triaxial accelerometers measure accelerations in the anterior-posterior, mediolateral, and longitudinal directions (10). Although uniaxial accelerometers are accurate in the prediction of EE during walking, triaxial accelerometers were found to be more suitable when a variety of different activities are involved (6, 17, 19, 33). However, the inclusion of heart-rate (HR) monitoring may improve the quality of the data collected by uniaxial accelerometry. The most widely used and extensively validated uniaxial accelerometer for assessment of PA among children is the ActiGraph (ActiGraph, Pensacola, FL) accelerometer (11, 15). The ActiTrainer is a recent ActiGraph uniaxial accelerometer with the capability to measure body acceleration and HR as well. On the other hand, the capability of the triaxial 3DNX (BioTel Limited, Bristol, UK) accelerometer to predict EE in free-living adult and adolescent cohorts has also been examined using the DLW method as a criterion measure (8). EE in free-living adult and adolescent cohorts has also been assessed using the triaxial ActiTrainer, HR, and 3DNX recording. The data from the two devices (18, 30). In addition, subjects were required to have concurrent ActiTrainer, HR, and 3DNX recording. The data from the two devices could not be exactly aligned because 3DNX is started manually by the investigator, whereas ActiTrainer is surrounded by a metal shield and packaged into a plastic enclosure measuring 50×40×15 mm, weights ~45 g including a 3-V (2,430 mAh) coin cell lithium battery, has a dynamic range of 0.25 to 2.5 g, a sampling frequency of 30 Hz, and contains a cantilevered rectangular piezoelectric bimorph plate and seismic mass, a charge amplifier, analog band-pass filters, and a voltage regulator to measure acceleration in a single axis. The filtered acceleration signals (in the longitudinal axis) generate counts that can estimate the magnitude of which is summed over a user-specific time (an epoch interval). At the end of each epoch, the summed value is stored in memory and

### Table 1. Descriptive characteristics of subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Means ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>6.9 ± 1.5</td>
<td>(4–10)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>24.7 ± 6.6</td>
<td>(11–48)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>122 ± 9.5</td>
<td>(100.5–140)</td>
</tr>
<tr>
<td>BML, kg/m²</td>
<td>16.6 ± 3.0</td>
<td>(13.6–26)</td>
</tr>
<tr>
<td>BMR, MJ/day</td>
<td>4.3 ± 0.6</td>
<td>(3.2–6.0)</td>
</tr>
<tr>
<td>TEE, MJ/day</td>
<td>6.6 ± 1.2</td>
<td>(3.9–10.3)</td>
</tr>
<tr>
<td>AEE, MJ/day</td>
<td>1.6 ± 0.6</td>
<td>(0.3–3.4)</td>
</tr>
<tr>
<td>PAL</td>
<td>1.5 ± 0.1</td>
<td>(1.2–1.8)</td>
</tr>
<tr>
<td>CPM-ActiTrainer</td>
<td>587 ± 121</td>
<td>(310–880)</td>
</tr>
<tr>
<td>CPM-3DNX xyz</td>
<td>816 ± 120</td>
<td>(562–1104)</td>
</tr>
<tr>
<td>Monitoring time, min</td>
<td>720 ± 46</td>
<td>(619–819)</td>
</tr>
</tbody>
</table>

Values are means ±SD; n = 49 (24 males; 25 females). BMI, body mass index; BMR, basal metabolic rate; TEE, total energy expenditure; AEE, activity-induced energy expenditure; PAL, physical activity level; CPM-ActiTrainer, counts per minute of uniaxial ActiTrainer accelerometer; CPM-3DNX xyz, counts per minute of triaxial sum of 3DNX accelerometer.

### Materials and Methods

**Subjects.** Ninety-six children were recruited from four validation centers at the universities of Glasgow (UK), Ghent (Belgium), Gothenburg (Sweden), and Zaragoza (Spain). The general protocol and main general findings have been described elsewhere (2). Forty-nine children from the initial cohort (25 female, 24 male) aged between 4–10 yr (mean age 6.9 ± 1.5 yr: Table 1) fulfilled the inclusion criteria for final data analysis, i.e., at least 6 days including day 0, subjects drank a weighed amount of $^3$H$_2$O resulting in an initial excess body water enrichment of 125–150 ppm for deuterium and 250–300 ppm for oxygen-18. Subsequent urine samples were collected from the second voiding in the morning and a subsequent voiding in the evening on days 1, 4, and 8. Urine samples were stored at −4°C in cryogenically stable tubes until analysis by gas chromatography (GC)–isotope ratio mass spectrometry. Samples were analyzed in duplicate for $^3$H$_2$O and $^3$H$_2$O at the Department of Human Biology at Maastricht University (Maastricht, The Netherlands). Carbon dioxide production rate was estimated from the differential disappearance of the two isotopes using equation A6 of Schoeller et al. (26) and was converted to EE using the de Weir equation (12), assuming an average diet resulting in a respiratory exchange ratio of 0.85 (3). The Schofield equations (27) based on gender, age, height, and weight were used to predict BMR for subjects aged 3–10 yr as described above: BMR (kcal/day) = 19.59 BM + 1.303 H + 414.9 (males) and BMR (kcal/day) = 16.969 BM + 1.618 H + 371.2 (females), where BM is body mass in kilograms and H is height in centimeters. These equations have been identified as those showing the best agreement with measured BMR in young children and adolescents (25).

**Accelerometry.** Free living daily PA levels and patterns were objectively assessed using the uniaxial ActiTrainer accelerometer (ActiGraph). The monitor was set to record PA in a 15-s epoch. The ActiTrainer is surrounded by a metal shield and packaged into a plastic enclosure measuring 50×40×15 mm, weights ~45 g including a 3-V (2,430 mAh) coin cell lithium battery, has a dynamic range of 0.25 to 2.5 g, a sampling frequency of 30 Hz, and contains a cantilevered rectangular piezoelectric bimorph plate and seismic mass, a charge amplifier, analog band-pass filters, and a voltage regulator to measure acceleration in a single axis. The filtered acceleration signals (in the longitudinal axis) generate counts that can estimate the magnitude of which is summed over a user-specific time (an epoch interval). At the end of each epoch, the summed value is stored in memory and...
the numerical integrator reset. The 3DNX model v3 (BioTel Limited, Bristol, UK; www.biotel.co.uk) is sensitive to movements in three planes: x (antero-posterior), y (mediolateral), and z (longitudinal). The unit measures $54 \times 54 \times 18$ mm and weighs 70 g including a 3.6-V lithium battery (Saft). Approximately 21 days of data can be stored when collecting at 15-s intervals. The unit contains two ADXL321 biaxial microelectromechanical (MEMS) sensors (Analog Devices, Surrey, UK) positioned orthogonally to measure acceleration in three movement planes.

Data for each axis were amplified and filtered (0.11 Hz high pass, 20 Hz low pass) to attenuate the DC responses and the sum of the rectified and integrated acceleration curves for the three axes measured.

Physical activity assessment. Subjects wore the accelerometers for 7 consecutive days between January and April 2009 during school term time simultaneous with the DLW measurements. Accelerometers were mounted on the right hip of each child on the same elastic belt and adjusted to ensure close contact with the body. However, accelerometer placement on the right hip was not randomized. In addition; all subjects were fitted with a HR transmitter belt (Suunto 16; Suunto Oy, Vantaa, Finland) on the chest to record HR continuously. The accelerometer outputs.

Sixty-one percent of the variation in AEE could be predicted by BM (partial $r^2 = 71\%$; $P < 0.05$), CPM-ActiTrainer (partial $r^2 = 11\%$; $P < 0.05$), and (ModHR – SedHR) (partial $r^2 = 4\%$; $P < 0.05$; Table 2). Similarly, 79% of the variance in TEE could be predicted by a model combining BM (partial $r^2 = 71\%$; $P < 0.05$) and CPM-3DNXxyz (partial $r^2 = 8\%$; $P < 0.05$) (Table 2). However, a model examining the predictive validity of the individual 3DNX axes indicated that 81% of the variance in the TEE could be predicted by BM (partial $r^2 = 71\%$; $P < 0.05$) and 3DNX (partial $r^2 = 10\%$; $P < 0.05$). Addition of 3DNX and 3DNX improved the model $R^2$ by 3%, which was not significant ($P > 0.05$; Table 2). The SEE of TEE estimate for ActiTrainer and 3DNX models ranged from 0.44 to 0.74 MJ/day or ~7–11% of the average TEE (Table 2). Height, age, and gender and other HR variables were not significant predictors of TEE in our cohort. Furthermore, adding quadratic terms of any of the predictors to any of the models did not result in significant improvement of fit. However, log transformation decreased model $R^2$ by approximately $\pm 2\%$. PE for all TEE models are shown in Table 2.

AEE prediction models using uniaxial and triaxial accelerometer outputs. Sixty-one percent of the variation in AEE could be explained by BM (partial $r^2 = 35\%$; $P < 0.05$), CPM-ActiTrainer (partial $r^2 = 22\%$; $P < 0.05$), and (ModHR – SedHR) (partial $r^2 = 4\%$; $P < 0.05$; Table 3). Similarly, 51% of the variance in AEE could be predicted by BM (partial $r^2 = 35\%$; $P < 0.05$) and CPD-xyz (partial $r^2 = 16\%$; $P < 0.05$; Table 3). However, a model examining the predictive validity of individual 3DNX axes indicated that 55% of the variance in AEE could be predicted by BM (partial $r^2 = 35\%$; $P < 0.05$) and 3DNX (partial $r^2 = 20\%$; $P < 0.05$). Addition of 3DNX and 3DNX improved the model $R^2$ by 3%, but this contribution was not significant ($P > 0.05$). The SE of AEE estimates ranged from 0.38 to 0.57 MJ/day or 24–26% of the average AEE for the CPM-ActiTrainer and CPM-3DNX models respectively (Table 3). Height, age, and gender were not significant predictors of AEE in our cohort. Furthermore, adding quadratic terms of any of the predictors to any of the models did not result in significant improvement of fit.
ever, log transformation improved model $R^2$ by $\leq 2\%$. PE for all AEE models are shown in Table 3.

**DISCUSSION**

EE can be estimated by measuring body acceleration (39). The DLW and indirect calorimetry that measures oxygen uptake, carbon dioxide production, and cardiopulmonary parameters are regarded as the gold-standard references of EE (39). Although accurate, gas analyzers for indirect calorimetry are expensive and they require specialized skills to operate (39). Therefore, accelerometers provide an alternative method of estimating EE in a free-living environment (4). In the current study, ActiTrainer with HR monitoring and 3DNX reported comparable model prediction accuracy for free-living TEE and AEE with similar models obtained with the Tracmor accelerometer in adults (5) based on reported SEE and partial $r^2$ values. Furthermore, Carter et al. (8) developed a model to predict TEE using as independent variables body height and activity counts as measured with the 3DNX accelerometer and found no association between accelerometer outputs and PAL, which is directly related to PA. In our study cohort on the other hand, uniaxial ActiTrainer and 3DNX, reported comparable validity relative to triaxial 3DNX accelerometer (Tables 2 and 3). Furthermore, there was significant positive association between ActiTrainer and 3DNX outputs and PAL (Fig. 2), which contradict the findings of Carter et al. (8) and is probably due to the fact that they used an earlier generation of the 3DNX accelerometer; lower subject numbers, i.e., 37 compared with 49 in the current study or sample-specific differences between the two studies.

A previous study in children indicated that activity counts from the CSA ActiGraph activity monitor contributed significantly to the explained variation in TEE and AEE in children (13). Similarly, the ActiTrainer ActiGraph accelerometer out-
puts were a significant predictor of EE in young children in the current study. Furthermore, addition of HR monitoring improved the predictive validity of the ActiTrainer. This contradicts the finding that activity measured using ActiGraph accelerometers is not related to AEE and PAL, and thus ActiGraph accelerometers may not be useful in predicting AEE in children (16). Examination of the validity of the models combining accelerometer outputs (CPM) and anthropometric measures to predict TEE, AEE, and PAL consistently indicated that uniaxial ActiTrainer outputs combined with HR monitoring were comparable to triaxial 3DNX outputs. However, comparison of our findings with previous prediction models is difficult because of differences in the activity profiles between study groups, different independent variables included in the regression such as fat free mass and sleeping metabolic rate, different age groups (5), and different devices used in other studies and other sample-specific differences.

This notwithstanding, use of accelerometer outputs to predict EE shows significant promise if accelerometer prediction accuracy of EE is improved by addressing specific issues related to accelerometer-based measurements of PA, such as intradividual variability in AEE (5), interindividual variability in accelerometer outputs in subjects performing standardized activities (33), and lack of comparability between accelerometer counts from different manufacturers (10). However, recently van Hees et al. (32) used the GENEa (Unilever Discover, UK) accelerometer that has capability to record raw accelerometer data in g units, and therefore, this device may be practical in attempts to standardize accelerometer outputs. Most devices, however, such as the ActiTrainer and 3DNX, used in this study do not have sufficient memory to store several days worth of raw data in g units. Furthermore, the raw data are already processed into counts using proprietary algorithms and technically there is no way of converting counts back to the original g units; hence interdevice comparison of counts may not be feasible.

According to Trost et al. (31), evidence indicates that some accelerometers may perform better than others under certain conditions, but the reported differences are not consistent or sufficiently compelling to single out one brand or type of accelerometer as being superior to the others. When it comes to selecting an accelerometer, issues of affordability, product reliability, monitor size, technical support, and comparability with other studies may be equally as important as the relative validity and reliability of an instrument. It is necessary to begin systematically evaluating the absolute and concurrent validity of these instruments under a variety of conditions. This can be accomplished only by comparing multiple instruments under the same conditions and against a suitable “gold standard” (33) since the relative validity and interinstrument reliability of a given accelerometry product is of primary importance. Moreover, accelerometer validation studies should report both \( r^2 \) and SEE values, as these two parameters complement each other and facilitate comparison across studies.

Uniaxial accelerometry with HR monitoring and triaxial accelerometry were comparable in the assessment of free-living EE in young children. This is consistent with previous studies (22, 33) but contradicts the findings of Plasqui et al. in adults (19), who reported that to measure the wide variety of daily life activities triaxial accelerometers are more suitable than uniaxial. Intuitively, it is expected that the sum of body accelerations in the three axes would be a better predictor of EE associated with motion. Therefore, triaxial accelerometers in general clearly provide more information that could provide

### Table 2. Comparison of the accuracy of TEE prediction models using body mass plus uniaxial with heart rate vs. triaxial accelerometry

<table>
<thead>
<tr>
<th>Model</th>
<th>( R^2 )</th>
<th>( \Delta r^2 )</th>
<th>SEE</th>
<th>PE</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td>71%</td>
<td>0.37</td>
<td>0.43</td>
<td></td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + ActiTrainer</td>
<td>82%</td>
<td>11%</td>
<td>0.49</td>
<td>0.28</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + ActiTrainer + (ModHR – SedHR)</td>
<td>86%</td>
<td>15%</td>
<td>0.44</td>
<td>0.23</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + 3DNXx</td>
<td>81%</td>
<td>10%</td>
<td>0.64</td>
<td>0.33</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + 3DNXx + 3DNXy</td>
<td>81%</td>
<td>10%</td>
<td>0.71</td>
<td>0.29</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + 3DNXx + 3DNXy + 3DNXz</td>
<td>83%</td>
<td>12%</td>
<td>0.71</td>
<td>0.30</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + ( \Sigma )3DNXxyz</td>
<td>79%</td>
<td>8%</td>
<td>0.74</td>
<td>0.29</td>
<td>( P &lt; 0.05 )</td>
</tr>
</tbody>
</table>

CPM-ActiTrainer, counts per minute of uniaxial ActiTrainer accelerometer contribution to the regression coefficient; CPM-3DNXx, counts per minute of \( z \)-axis (longitudinal) contribution to the regression coefficient; CPM-3DNXy, counts per minute of \( x \)-axis (anteroposterior) contribution to the regression coefficient; CPM-3DNXz, counts per minute of \( y \)-axis (mediolateral) contribution to the regression coefficient; CPM-\( \Sigma \)3DNXxyz, counts per minute of triaxial sum of 3DNX accelerometer contribution to the regression coefficient; (ModHR – SedHR), difference in HR during sedentary and moderate activities; \( \Delta r^2 \), change in \( R^2 \) relative to the first model containing body mass as the single predictor; TEE, total energy expenditure; BM, body mass; SEE, standard error of estimate; PE, prediction error.

### Table 3. Comparison of the accuracy of AEE prediction models using body mass plus uniaxial accelerometry and heart rate vs. triaxial accelerometry

<table>
<thead>
<tr>
<th>Model</th>
<th>( R^2 )</th>
<th>( \Delta r^2 )</th>
<th>SEE</th>
<th>PE</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td>35%</td>
<td>0.28</td>
<td>0.26</td>
<td></td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + ActiTrainer</td>
<td>57%</td>
<td>22%</td>
<td>0.4</td>
<td>0.18</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + ActiTrainer + (ModHR – SedHR)</td>
<td>61%</td>
<td>26%</td>
<td>0.38</td>
<td>0.18</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + 3DNXx</td>
<td>55%</td>
<td>20%</td>
<td>0.5</td>
<td>0.20</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + 3DNXx + 3DNXy</td>
<td>56%</td>
<td>21%</td>
<td>0.56</td>
<td>0.19</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + 3DNXx + 3DNXy + 3DNXz</td>
<td>58%</td>
<td>22%</td>
<td>0.57</td>
<td>0.19</td>
<td>( P &lt; 0.05 )</td>
</tr>
<tr>
<td>BM + ( \Sigma )3DNXxyz</td>
<td>51%</td>
<td>16%</td>
<td>0.56</td>
<td>0.20</td>
<td>( P &lt; 0.05 )</td>
</tr>
</tbody>
</table>
marginal improvements in validity in other samples (e.g., older children, more active, more diverse/complex activities besides occasional walking). However, this was not the case for the 3DNX accelerometer in our cohort, since only the longitudinal axis was a significant predictor of EE.

This study had several limitations; firstly, EE and PA are distinct constructs, which may limit attempts to validate PA measures against EE (28). Consequently, correlation between accelerometer output and DLW-derived EE measures, such as AEE or PAL are often poor and mainly determined by the subject’s characteristics such as BMI, age, sex, and height (19) and even the activity profiles of the study population. Secondly, it is unclear how the performance of uniaxial and triaxial accelerometer observed in the present study may generalize in other settings since they may be sample or device specific; thus further enquiry is warranted in other free-living populations.

To conclude, hip-mounted uniaxial accelerometer with HR monitoring and triaxial accelerometer have comparable validity in assessing free-living EE in young children. Uniaxial ActiTraining and triaxial 3DNX were valid for assessing EE in young children. Furthermore, addition of HR monitoring improved the predictive validity of accelerometry. However, there is a wide array of activity monitors that have yet to be properly validated, and therefore, accuracy of most remains to be determined.

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STATEMENT OF ETHICS
We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Approval by the appropriate Ethics Committees was obtained by each of the four centers at the universities of Glasgow (UK), Ghent (Belgium), Gothenburg (Sweden), and Zaragoza (Spain) doing the field work. Study children did not undergo any procedure before both they and their parents had given consent for examinations, collection of samples, subsequent analysis, and storage of personal data and collected samples.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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