

Statistical analysis

All the analyses were performed using SPSS 16.0 J for Windows (IBM Japan). The characteristics of the subjects are expressed as means and standard deviations. As the PA data were not normally distributed, they were expressed as both means and standard deviations and medians with ranges. Differences between grades and sexes were analysed by two-way ANOVA. The linear relationships between PAL and light PA, moderate PA, vigorous PA and MVPA were assessed using Pearson's correlation coefficients. Student's paired *t* test, Spearman's rank correlation coefficients, intraclass correlation coefficient and Bland–Altman plot analysis⁽²²⁾ were used to compare data obtained from the DLW method and accelerometry.

Results

The physical characteristics of the subjects are shown in Table 1. Height and weight were significantly lower in girls than in boys (both $P < 0.001$). The percentage of body fat was higher in girls than in boys ($P < 0.001$). TEE_{DLW} and RMR_m were significantly lower in girls than in boys. PAL_{DLWm} ranged from 1.48 to 2.54 in individual subjects, with a mean of 1.91. PAL_{DLWm} was not different between sexes or grades. All subjects included in the analysis wore the accelerometers for more than four weekdays and more than one weekend day. Of the subjects, 82% wore the accelerometer for five weekdays, and 90.2% wore it for two weekend days. TEE_{DLW} and PAL_{DLWm} were not different between the subjects included in the analysis and subjects excluded due to insufficient accelerometer use (TEE_{DLW} 11.0 (SD 2.6) *v.* 11.0 (SD 2.5) MJ/d; RMR_m 5.7 (SD 0.9) *v.* 5.9 (SD 1.1) MJ/d; PAL_{DLWm} 1.91 (SD 0.30) *v.* 1.85 (SD 0.26), respectively).

Of the present subjects, 75% (twenty-seven boys and ten girls) participated in exercise other than the physical education class. The frequency and duration of exercise were 3.6 (SD 1.8) times/week and 523 (SD 291) min/week for boys, and 2.1 (SD 1.1) times/week and 318 (SD 219) min/week for girls, respectively. Major sports activities included kendo (n 5), soccer (n 4), tennis (n 4), basketball (n 4), track and field (n 4) and baseball (n 3) for boys, and badminton (n 6), track and field (n 5) and tennis (n 3) for girls. The percentage of body fat was significantly lower in subjects who exercised; however, RMR_m did not differ with exercise activity (Table 2). Non-wear time, including bathing, periods of PA preventing accelerometer use and times when participants forgot to wear the accelerometer, also did not differ with exercise activity. TEE_{DLW} , TEE_{ACcm} and TEE_{ACcp} were 11.0 (SD 2.0), 10.3 (SD 1.9) and 10.7 (SD 2.1) MJ/d for all subjects, respectively. All three indices were significantly greater in subjects who exercised compared with subjects who did not exercise. Walking step counts were greater in subjects who exercised; however, TEE_{ACcp} did not differ with exercise activity.

RMR_m and RMR_p correlated strongly in all subjects and in subjects who exercised, although RMR_p was significantly greater than RMR_m in all subjects and subjects who did not exercise (Fig. 1; Table 3). The percentage difference between

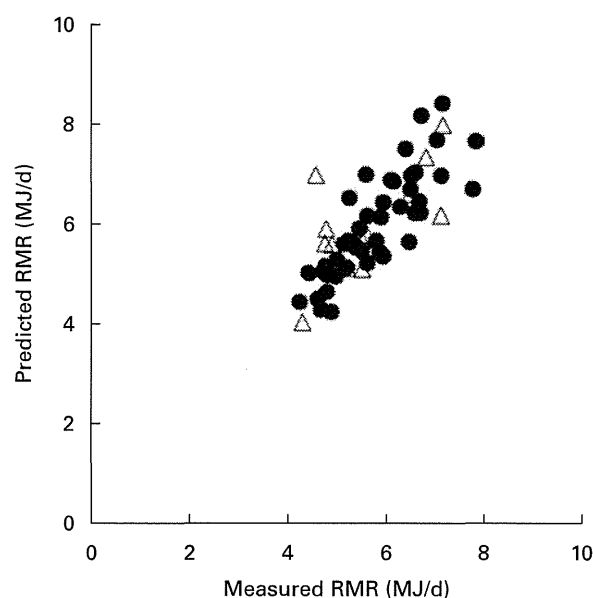


Fig. 1. Correlation between measured and predicted RMR. ●, Subjects who exercised; Δ, subjects who did not exercise. The Spearman's correlation coefficients were 0.732 ($P < 0.001$) for all subjects, 0.800 ($P < 0.001$) for subjects who exercised, and 0.436 ($P = 0.020$) for subjects who did not exercise.

RMR_m and RMR_p correlated with body weight (r 0.401, $P = 0.002$) and the percentage of body fat (r 0.524, $P < 0.001$), but not with sex, age or height. TEE_{DLW} correlated significantly with both TEE_{ACcm} and TEE_{ACcp} , although TEE_{ACcp} was significantly smaller than TEE_{DLW} in all the subjects. In subjects who exercised, accelerometry underestimated TEE significantly using either RMR_m or RMR_p . Spearman's rank correlation coefficients and intraclass correlation coefficient were lower for PAEE than for TEE in all subjects and in the exercising and non-exercising subjects. Accelerometry underestimated PAEE significantly in comparisons between $PAEE_{DLWm}$ *v.* $PAEE_{ACcm}$, and $PAEE_{DLWm}$ *v.* $PAEE_{ACcp}$ in all subjects and in subjects who exercised. Subjects who did not exercise showed a weaker correlation between PAEE assessed by the DLW method and accelerometry. In comparison with PAL, Spearman's rank correlation coefficient and intraclass correlation coefficient were lower than those for PAEE and TEE. Accelerometry underestimated PAL only when PAL_{ACcp} was compared with PAL_{DLWm} in all subjects and in exercising and non-exercising subjects. The percentage of body fat correlated significantly with the percentage difference in TEE, PAEE and PAL assessed by the DLW method and accelerometry using RMR_p (r 0.304, $P = 0.018$ for TEE and PAL; r 0.349, $P = 0.006$ for PAEE), and PAL using RMR_m (r 0.304, $P = 0.018$). Sex, age, body weight and height did not correlate significantly with the percentage difference between TEE, PAEE and PAL. The Bland–Altman agreement plots showed a moderate negative correlation between PAL assessed by either the DLW method or accelerometry, even when measured or RMR_p was used (Fig. 2).

When the subjects were divided into tertile subgroups for PAL, 47, 45 and 52% of the subjects were stratified into the same tertiles of PAL_{DLWm} *v.* PAL_{ACcm} , PAL_{DLWp} *v.* PAL_{ACcp}

Table 3. Comparison of energy expenditure measured by the doubly labelled water method and triaxial accelerometry

	All subjects (n 60)						Subjects who exercised (n 45)						Subjects who did not exercise (n 15)								
	%Δ*	SD	P†	Cor†	P‡§	ICC	95% CI	%Δ*	SD	P†	Cor†	P‡§	ICC	95% CI	%Δ*	SD	P†	Cor†	P‡§	ICC	95% CI
RMR _m v. RMR _p	4.5	11.4	0.005	0.792	<0.001	0.770	0.622, 0.861	2.4	9.1	0.174	0.800	<0.001	0.782	0.603, 0.887	6.9	13.3	0.001	0.436	0.020	0.318	-0.018, 0.602
TEE _{DLW} v. TEE _{ACCm}	-4.0	13.3	0.003	0.783	<0.001	0.730	0.556, 0.837	-5.6	13.5	0.034	0.778	<0.001	0.751	0.581, 0.856	6.7	20.1	0.386	0.512	0.051	0.513	0.028, 0.804
TEE _{DLW} v. TEE _{ACCp}	-0.7	15.8	0.193	0.741	<0.001	0.730	0.587, 0.829	-3.1	13.5	0.002	0.758	<0.001	0.681	0.431, 0.824	0.5	12.0	0.887	0.826	<0.001	0.832	0.567, 0.941
PAEE _{DLWm} v. PAEE _{ACCm}	-4.3	33.1	0.003	0.551	<0.001	0.452	0.218, 0.636	-5.1	32.5	0.013	0.522	0.002	0.394	0.074, 0.645	-3.3	34.4	0.098	0.196	0.318	0.041	-0.300, 0.388
PAEE _{DLWp} v. PAEE _{ACCp}	-11.8	61.3	0.193	0.507	<0.001	0.467	0.247, 0.643	0	38.8	0.071	0.500	0.004	0.487	0.183, 0.709	26.3	78.3	0.997	-0.089	0.654	-0.147	-0.510, 0.245
PAEE _{DLWm} v. PAEE _{ACCp}	-2.8	34.1	0.008	0.557	<0.001	0.508	0.289, 0.676	-6.2	29.4	0.011	0.585	<0.001	0.545	0.234, 0.752	1.1	38.9	0.249	0.121	0.540	-0.033	-0.390, 0.335
PAL _{DLWm} v. PAL _{ACCm}	-0.7	15.8	0.298	0.426	0.001	0.361	0.121, 0.562	-3.1	13.6	0.077	0.594	0.001	0.545	0.255, 0.747	2.0	18.4	0.931	0.218	0.265	0.105	-0.289, 0.461
PAL _{DLWp} v. PAL _{ACCp}	-0.7	15.8	0.163	0.412	0.001	0.351	0.113, 0.553	-3.1	13.0	0.054	0.514	0.003	0.488	0.183, 0.710	2.0	18.4	0.878	0.082	0.678	-0.021	-0.405, 0.357
PAL _{DLWm} v. PAL _{ACCp}	-4.6	13.4	0.002	0.391	0.002	0.275	0.039, 0.486	-5.0	11.9	0.009	0.551	0.001	0.445	0.121, 0.683	-4.2	15.1	0.077	0.028	0.888	-0.095	-0.411, 0.262

ICC, interclass correlation coefficient; RMR_m, measured RMR; RMR_p, predicted RMR; TEE_{DLW}, total energy expenditure measured by the doubly labelled method; TEE_{ACCm}, total energy expenditure measured by accelerometry using RMR_m; TEE_{ACCp}, total energy expenditure measured by accelerometry using RMR_p; PAEE_{DLWm}, physical activity energy expenditure using TEE_{DLW} × 0.9 - RMR_m; PAEE_{ACCm}, physical activity energy expenditure using TEE_{ACCm} and RMR_m (TEE_{ACCm} × 0.9 - RMR_m); PAEE_{DLWp}, physical activity energy expenditure using TEE_{DLW} × 0.9 - RMR_p; PAEE_{ACCp}, physical activity energy expenditure using TEE_{ACCp} and RMR_p (TEE_{ACCp} × 0.9 - RMR_p); PAL_{DLWm}, physical activity level using TEE_{DLW} and RMR_m (TEE_{DLW}/RMR_m); PAL_{ACCm}, physical activity level using TEE_{ACCm} and RMR_m (TEE_{ACCm}/RMR_m); PAL_{DLWp}, physical activity level using TEE_{DLW} and RMR_p (TEE_{DLW}/RMR_p); PAL_{ACCp}, physical activity level using TEE_{ACCp} and RMR_p (TEE_{ACCp}/RMR_p); *%Δ: (data measured by accelerometry - data measured by the doubly labelled method)/(data measured by the doubly labelled method) × 100 or (RMR_p - RMR_m)/RMR_m × 100.

† P: P value for the paired t test.

‡ Cor: Spearman's rank correlation coefficient.

§ P‡: P value for Spearman's rank correlation coefficient.

and PAL_{DLWm} v. PAL_{ACCp}. However, 27, 30 and 25% of the subjects were divided into the lower PAL subgroups according to PAL_{DLWm} v. PAL_{ACCm}, PAL_{DLWp} v. PAL_{ACCp} and PAL_{DLWm} v. PAL_{ACCp}.

The average number of walking steps and the duration of light PA, moderate PA, vigorous PA and MVPA assessed by accelerometry were 14 132 (SD 3469) steps/d, and 894 (SD 66), 89 (SD 29), 13 (SD 14) and 103 (SD 39) min/d, respectively. MVPA in subjects who did or did not exercise was 113 (SD 38) and 72 (SD 22) min/week, respectively. MVPA in weekends was significantly shorter (*P*<0.001) than in weekdays (81 (SD 47) v. 111 (SD 42) min/d). Walking step counts were also significantly lower (*P*<0.001) in weekends (10 630 (SD 5622) steps/d) compared with weekdays (15 652 (SD 3632) steps/d). MVPA correlated significantly with PAL_{DLWm} (*r* 0.341, *P*=0.008). The linear relationship between MVPA and PAL_{DLWm} (PAL_{DLWm} = MVPA × 0.003 + 1.65) showed that 30 min of MVPA per d was equivalent to 1.74 of PAL_{DLW}.

Discussion

This is the first study to assess PA among Japanese junior high school students using the DLW method and triaxial accelerometry. TEE assessed by accelerometry was found to have good accuracy as determined by comparison with TEE_{DLW}, whereas the accuracy of PAEE and PAL was lower compared with that of TEE. The errors caused by accelerometry were considered to be attributable to the error in the prediction of RMR and the assessment of exercise intensity.

The average height of the subjects in the present study was slightly higher, and the girls' height slightly lower than that in data collected in 2010 by the Ministry of Education, Culture, Sports, Science, and Technology, Japan⁽¹⁾. In the present study, only three students weighed more than 120% of the standard body weight for sex, age and height in Japanese students⁽¹⁾.

The average PAL_{DLWm} of 1.91 (SD 0.30) in the present study was greater than that in previous studies conducted in Western countries (PAL 1.48-1.89)⁽²³⁻³³⁾. The proportion of the present subjects performing exercise, with the exception of the physical education class, was higher than that in a previous report on the entire student population at this school (in the previous study, boys 70% and girls 46%)⁽³⁴⁾. As the subjects who exercised participated in MVPA about 2 h each week and had a smaller percentage of body fat than non-exercising subjects, they were well trained and had higher energy expenditure.

The average number of walking steps in the present study was also greater than that in previous studies that used uniaxial accelerometry. The accuracy of step counts in the present accelerometry had already been examined in an adult population only, and it is possible that there may be differences in accuracy between adult and children⁽³⁵⁾. The accelerometer used in the present study underestimated step counts by 18% when subjects walked 55 m/min with a normal step frequency. However, this accelerometry did not show significant differences in step count compared with visually counted step

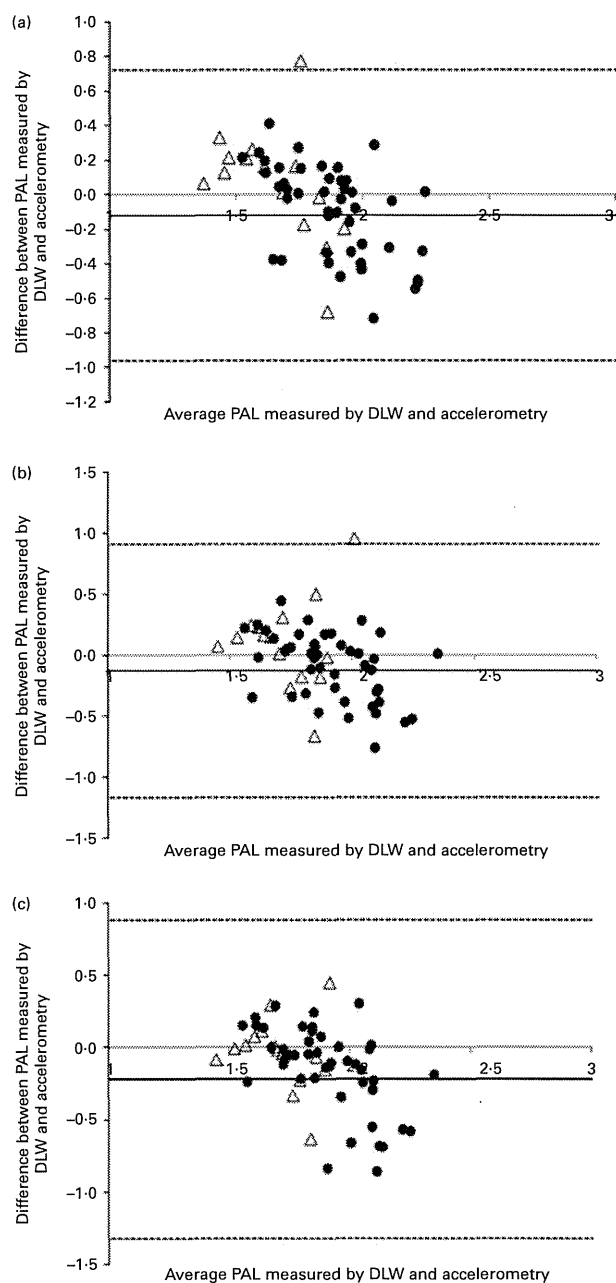


Fig. 2. Bland–Altman plots of physical activity level (PAL) assessed by either the doubly labelled water (DLW) method or an accelerometer. ●, Subjects who exercised; △, subjects who did not exercise. —, Mean PAL measured by the DLW method and accelerometry; ---, mean (2 sd) of PAL measured by the DLW method and accelerometry. Comparison of (a) PAL measured by the DLW method and accelerometry with predicted RMR ($r = 0.564$, $P < 0.001$), (b) PAL measured by the DLW method and accelerometry with the measured RMR ($r = 0.381$, $P = 0.003$) and (c) PAL measured by the DLW method with the measured RMR and accelerometry with the predicted RMR ($r = 0.508$, $P < 0.001$).

counts at walking speeds of 75 and 95 m/min. Although we could not examine the accuracy of assessing MVPA by accelerometry, the relationship between MVPA and the number of walking steps was similar to that reported in previous studies. Tudor-Locke *et al.*⁽³⁶⁾ recommended 10 000–11 700 steps/d for adolescents to satisfy 60 min of MVPA. In the

present study, 60 min of MVPA was equivalent to an average of 11 006 steps/d.

The present study shows that TEE measured by EW4800P triaxial accelerometry had good accuracy. The percentage difference between TEE_{DLW} and TEE_{ACCP} was -0.7 (SD 15.8)%. Although the commercially available software uses RMR_p , in the present study, we also used RMR_m to calculate energy expenditure. TEE_{ACCP} showed less difference than TEE_{ACCM} in comparison with TEE_{DLW} . The difference between TEE_{DLW} and TEE_{ACCP} was very close to the results of a previous study that used the same accelerometer in elderly subjects, and showed a 1.6% difference⁽⁹⁾. These results suggest that this accelerometer can evaluate TEE with a similar level of accuracy in both elderly and junior high school students, at least at the group level. In addition, this accelerometer has very good accuracy compared with other accelerometers. A study using triaxial accelerometry in an age group similar to that in the present study and the most accurate estimation equation⁽³⁷⁾ showed that the root mean square error was 40.72% for boys and 59.72% for girls. We examined the effects of sex, age, body weight, height and percentage of body fat on the difference between TEE_{DLW} and TEE_{ACCP} or TEE_{ACCM} , and found only the percentage of body fat to be correlated significantly with the percentage difference between TEE_{DLW} and TEE_{ACCP} . As the percentage of body fat also correlated with the difference between RMR_m and RMR_p , the prediction error in RMR for subjects with higher body fat deposition affected the estimation error of TEE using RMR_p .

Although TEE_{ACCP} and TEE_{ACCM} showed good accuracy as established by comparison with TEE_{DLW} , RMR accounted for a large portion of TEE in the study subjects. To lessen the contribution of RMR, we compared PAEE and PAL measurements obtained using the two methods. The accuracy for PAEE and PAL was lower than that for TEE. For PAEE, the difference between the two methods was most apparent when RMR_p was used in both the DLW method and accelerometry. In particular, the mean difference in PAEE and PAL was overestimated by accelerometry in subjects who did not exercise. The reasons for this finding were that one subject who did not exercise showed a large overestimation of PAEE by accelerometry, while the estimation error of PAEE by accelerometry was relatively small in the other non-exercising subjects. In comparison with PAL, PAL_{DLWm} and PAL_{ACCM} showed the strongest correlation. One reason for these results is the prediction error of RMR. RMR_p is based on a standard value of RMR for Japanese in the dietary reference intake for Japan. The dietary reference intake for Japan is revised every 5 years, although the standard RMR value for children was calculated using the data collected in the 1950s. When the dietary reference intake was revised in 2010 based on data measured in the 2000s, the standard value of RMR in females aged 18–29 years decreased from 98.7 to 92.5 kJ/d. It is therefore possible that RMR_p may also be overestimated in adolescents. Another possible reason for the overestimation of RMR_p was the systematic error of the measurement. Cooper *et al.*⁽³⁸⁾ suggested that there was a systematic error in calorimetry systems. The system used in the present study



has been tested previously⁽²¹⁾. As the standard value was based on data collected more than 50 years ago, we could not examine the systematic error between the present system and systems that were used to decide the standard value of RMR. Given that RMR can vary with age, maturation, body weight and the level of PA, we considered that a better estimation of PAEE may be obtained using a more accurate measurement of RMR⁽³⁹⁾.

In the present study, subjects who exercised tended to have a greater underestimation of PAL by accelerometry than other subjects who did not exercise. RMR and non-wear time of accelerometry were not different between the exercising and non-exercising subjects, while walking step counts were greater in subjects who exercised. As most accelerometers are more sensitive for accelerations in the vertical plane and less sensitive for a more complex movement⁽⁴⁰⁾, the intensity of exercise may be estimated less accurately. In addition, although the prediction model of EW4800P was constructed using data from adults, the models are population specific⁽⁴¹⁾. It is therefore possible that PA in some adolescents may not have been estimated correctly.

The most significant limitation of the present study was that all the subjects were recruited from one school. Most of the students in the present study used trains to go to school, and had to walk approximately 15 min each way from the nearest station to the school. In Japan, most public school students go to school on foot or by a bicycle. It is therefore necessary to collect data from other schools to generalise the present results to other Japanese junior high school students.

Second, although we sent an information letter to all the students, the participation rate was quite low. We are therefore not sure whether the subjects in the study were representative of the students in this school.

Third, some subjects had issues with improper wearing of the accelerometer. According to our criteria, approximately 25% of the subjects were excluded from the analysis because of inappropriate accelerometer use. Of the seventeen subjects excluded from the analysis, fifteen did not wear the accelerometer on any weekend days. All the subjects included in the analysis wore the accelerometer more than 12 h/d and for more than 5 d. This exclusion rate was not greater than that in the International Children's Accelerometry Database, which found that only 62.2% of boys and 59.3% of girls in their study wore an accelerometer more than 12 h/d over periods longer than 4 d⁽⁴²⁾. On weekdays, members of the research team met with the subjects every morning to remind them to wear their accelerometers. However, TEE_{DLW} and PAL_{DLW} were not different between the subjects included in the analysis and those who were excluded.

Fourth, we used a triaxial accelerometer made by a Japanese company. This made it difficult to compare the present results with those from Western countries, which have most often used Actigraph accelerometers. Actigraph accelerometers are not used widely in Japan, and Japanese people tend to be more familiar with pedometers and accelerometers made by Japanese companies. The present study examined

the accuracy of the EW4800P accelerometer, and showed that it can estimate the total PA with a high degree of accuracy.

In conclusion, based on a comparison with the DLW method, the present study showed that EW4800P triaxial accelerometry can estimate daily TEE with good accuracy and precision. However, the accuracy of PAEE and PAL estimations was not high in Japanese adolescents. Prediction of RMR in Japanese adolescents and the prediction model of accelerometry for adolescents therefore need to be improved.

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The relationship of body composition to daily physical activity in free-living Japanese adult men

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Abstract

The objective of the present study was to investigate whether a previously reported apparent negative relationship between fat mass and daily physical activity in Japanese adult women would also be observed in Japanese adult men. The subjects were grouped into quartiles of BMI and body fat percentage (%BF). The number of steps walked each day and the duration of light- to vigorous-intensity physical activity were assessed by an accelerometer over the same period of time as for the doubly labelled water experiment. The results showed that BMI negatively correlated with the number of steps and time spent in moderate-intensity physical activity, whereas %BF showed a negative relationship with physical activity-related energy expenditure (PAEE)/body weight (BW) and physical activity level. The analysis of data using %BF quartiles revealed that PAEE/BW decreased from the second quartile in which the BMI was $<25 \text{ kg/m}^2$. These observations are similar to those reported in our previous study in Japanese adult women. These cross-sectional studies cannot prove causality, and that obesity causes physical inactivity may be the case. However, the results of the present study provide information regarding which physical activity variables should be used in longitudinal studies.

Key words: Body composition: Physical activity: Doubly labelled water: Accelerometry: Japanese adult men

The prevalence of obesity is rising rapidly in most Asian countries, especially in Japan with an increase of 46% from 1976–80 to 2000⁽¹⁾. Specifically, the overall prevalence of overweight and obese adult men in Japan has increased gradually over the past 20 years, whereas the prevalence of overweight and obese adult women has not changed⁽²⁾. Obesity is commonly caused by an energy imbalance, with low total energy expenditure (TEE) possibly being a contributing factor in this process. While a negative relationship between obesity and daily physical activity has been reported^(3,4), the majority of studies have not observed this relationship^(5,6). Therefore, the relationship between BMI

(body weight (BW, kg) divided by height squared (m^2)) and daily physical activity still remains unclear.

In studies using the doubly labelled water (DLW) method, the most accurate method for measuring TEE in free-living conditions^(7,8), the majority of Western adult populations showed no relationship between BMI and physical activity level (PAL, the ratio of TEE:RMR)^(9–11).

On the other hand, we have recently reported that Japanese adult women with higher fat deposition had higher body fat percentage (%BF) with an apparent lower PAL compared with those with lower fat deposition⁽¹²⁾. However, this association was not observed when women were categorised using BMI. In addition,

Abbreviations: %BF, body fat percentage; BW, body weight; DLW, doubly labelled water; FM, fat mass; FFM, fat-free mass; PAEE, physical activity-related energy expenditure; PAL, physical activity level; TEE, total energy expenditure.

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step counts or time spent in moderate- or vigorous-intensity physical activity assessed by an accelerometer (i.e. the part composed of PAL) was also found to be lower in women with higher fat deposition⁽¹²⁾.

However, Westerterp & Goran⁽¹³⁾ using a meta-analysis of data of 290 adults from twenty-two DLW studies reported that a higher PAL was related to a lower fat mass (FM) only in men but not in women. This indicates that the relationship between physical activity and fat deposition may be different between the sexes. However, this relationship was not observed if data were stratified according to BMI.

The main aim of the present study was to examine whether the negative relationship that we observed between %BF and daily physical activity in our previous cross-sectional study in Japanese adult women⁽¹²⁾ was also apparent in Japanese adult men. Information from the present study will help to understand the role of daily physical activity in the increased prevalence of obesity in adult males.

Methods

Subjects

Participants were recruited through health-care centres or at workplaces in three urban districts in central and western Japan, as described previously⁽¹²⁾. In each location, subjects were included for the study according to the following criteria: (1) in good health; (2) not involved in hard physical labour such as farming or athletics; (3) BMI > 18.5 kg/m²; (4) living in their home prefecture 2 weeks before and during the study; (5) not on a weight-loss or treatment diet; (6) alcohol consumption < 40 g/d. The occupations of the participants were mainly salesmen, teachers, clerks and desk jobs, and some were unemployed. Overall, eighty-five male subjects aged 30–69 years were selected for the study. Over the entire assessment period, subjects were instructed to carefully maintain their normal daily activities and eating patterns and to make no conscious effort to lose or gain weight. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethical Committee of the National Institute of Health and Nutrition in Japan. Written informed consent was obtained from all subjects.

Experimental procedures

The experimental design has been described in detail in our previous study⁽¹²⁾. Briefly, urine samples were collected and BW and height measured on the day before the assessment of physical activity (day 0). RMR was measured in the supine position using a Douglas bag in the early morning 12 h or longer after the last meal. A single dose of DLW was then given orally to each subject. After administration of this dose, the participants were instructed to collect urine samples on the following day and at eight additional times at the same time of the day during the 2-week study period. On day 15, BW and height were measured again to examine changes in BW during the study period. The subjects were then provided with an accelerometer and a self-administered diet history

questionnaire. Daily physical activity was estimated over the same 2-week study period under free-living conditions using the DLW method and accelerometer.

Measurement of energy expenditure and body composition

We measured TEE using the DLW method, as described previously⁽¹²⁾. Briefly, the single dose of DLW consisted of approximately 0.06 g/kg BW of ²H₂O (99.8 atom%; Cambridge Isotope Laboratories) and 1.4 g/kg BW of H₂ ¹⁸O (10.0 atom%; Taiyo Nippon Sanso). Isotopic enrichment of urine samples was measured using an isotope ratio mass spectrometer (model DELTA Plus; Thermo Electron Corporation). The ²H and ¹⁸O zero-time intercepts and elimination rates (k_H and k_O) were calculated using least-squares linear regression of the natural logarithm of the isotope concentration as a function of the elapsed time from dose administration. The zero-time intercepts were used to determine isotope pool sizes. Total body water was calculated as the mean value of the isotope pool size of ²H divided by 1.041 and that of ¹⁸O divided by 1.007. Fat-free mass (FFM) was calculated by assuming a hydration of 0.732⁽¹⁴⁾. FM was calculated as BW minus FFM, while %BF was computed from BW and FFM. Calculation of TEE (kJ/d) was performed using the modified Weir formula⁽¹⁵⁾ based on the CO₂ production rate and respiratory quotient. Food quotient calculated from the diet history questionnaire was used instead of the respiratory quotient. This assumes that under conditions of perfect nutrient balance, the food quotient must equal the respiratory quotient^(16,17). PAL was estimated by dividing TEE by RMR. Physical activity-related energy expenditure (PAEE) was calculated as 0.9 × TEE – RMR, assuming that the thermic effect of food was 10% of TEE⁽¹⁸⁾.

Other measurements

Measurements of anthropometry, accelerometry and the diet history questionnaire have been described in detail in our previous study⁽¹²⁾. Briefly, a uniaxial accelerometer (Lifecorder EX; Suzuken Company Limited) was used for measuring the intensity of physical activity and step counts. The intensity of physical activity every 2 min was classified as either light (<3 metabolic equivalents), moderate (3 to <6 metabolic equivalents) or vigorous (≥6 metabolic equivalents)⁽¹⁶⁾. The validation and methodology of the diet history questionnaire have been described in detail elsewhere⁽¹⁷⁾.

Statistical analysis

Data are presented as means and standard deviations. BMI was calculated as BW (measured before the DLW dose) divided by height squared (kg/m²). The associations between physical activity and BMI or %BF, expressed as continuous variables, were examined by the linear regression analysis with or without adjustment for covariates. Subjects were then grouped according to quartiles of BMI and %BF. A one-way ANOVA or ANCOVA was used to compare the variables in the quartiles, with Fisher's least-square difference used as

Table 1. Participants' characteristics, energy expenditure components and physical activity variables, grouped according to BMI||
(Mean values and standard deviations)

BMI quartiles...	First (18.7–22.5 kg/m ²)		Second (22.6–24.5 kg/m ²)		Third (24.6–27.5 kg/m ²)		Fourth (27.6–39.1 kg/m ²)		P (ANOVA)	r [†]
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Energy expenditure										
TEE (kJ/d)	11 070	1290	11 663	2055	12 188	1949	13 396†††‡§	2172	0.009	0.458**
RMR (kJ/d)	5629	769	5792	774	6039	809	7165†††‡§§	861	<0.001	0.658**
PAL	1.99	0.23	2.02	0.29	2.03	0.29	1.87	0.19	0.227	-0.168
PAEE (kJ/d)	4335	957	4705	1521	4930	1454	4891	1390	0.830	0.169
PAEE/BW (kJ/d per kg)†††	71.7	16.9	69.0	21.8	67.4	18.9	52.9†††§	12.2	0.008	-0.343**
Accelerometer										
Step counts (per d)	10 296	2699	10 085	2891	9848	3001	7992††§	3222	0.027	-0.259*
Light, <3 MET (min/d)	56.6	12.5	55.1	16.2	57.6	20.2	54.9	21.4	0.695	-0.017
Moderate, 3 to <6 MET (min/d)	39.8	17.9	37.5	12.9	35.8	19.6	24.2†††‡§	14.3	0.009	-0.298**
Vigorous, 6 MET (min/d)	3.6	3.0	4.7	6.6	3.2	4.0	2.2	4.6	0.397	-0.154

TEE, total energy expenditure; PAL, physical activity level (TEE/RMR); PAEE, physical activity-related energy expenditure (0.9 × TEE – RMR); BW, body weight; MET, metabolic equivalents.

There was a significant correlation with BMI: * $P < 0.05$, ** $P < 0.01$.

Mean values were significantly different from those of the first quartile: † $P < 0.05$, †† $P < 0.01$.

Mean values were significantly different from those of the second quartile: ‡ $P < 0.05$.

Mean values were significantly different from those of the third quartile: § $P < 0.05$.

|| The subjects were categorised by quartile. The number of subjects in each quartile was twenty-five. In the nine intensity levels (1–9) of physical activity assessed by using an accelerometer, 1–3 indicates light intensity, 4–6 moderate intensity and 7–9 vigorous intensity.

† The associations between BMI and physical activity variables and energy expenditure components, expressed as continuous variables, were examined by the linear regression analysis.

|||| A statistical significance in TEE among the quartiles was still found even after adjustment for BW using ANCOVA ($P = 0.026$).

††† There was no significant difference in PAEE among the quartiles after adjustment for BW using ANCOVA ($P = 0.155$).

||||| There was no significant difference between BMI and PAEE after adjustment for BW (partial $r = 0.046$, $P = 0.677$).



a *post hoc* test for multiple comparisons. Differences were considered statistically significant when the *P* value was <0.05. All statistical analyses were carried out using SPSS for Windows (version 16.0 J; SPSS, Inc.).

Results

Of the total eighty-five men studied, the proportion of normal-weight (BMI 18.5 to <25 kg/m²), overweight (BMI ≥25 to <30 kg/m²) and obese participants (BMI ≥30 kg/m²) was 54.1, 35.3 and 10.6%, respectively. The mean age of the subjects was 47.5 (SD 11.0, range 30–69) years. The mean BW, height and BMI were 73.5 (SD 14.0, range 51.1–116.3) kg, 1.70 (SD 0.07, range, 1.51–1.88) m and 25.3 (SD 3.9, range 18.7–39.1) kg/m², respectively. BW did not change during the study (change in BW: -0.13 (SD 0.04) kg, *P*=0.987). The range of PAL was 1.46–2.51 with a mean value of 1.98.

The relationships between BMI and energy expenditure or physical activity variables are shown in Table 1 and Fig. 1. Both FM (*r* 0.824) and FFM (*r* 0.714) showed a linear correlation with BMI. TEE increased linearly with BMI, whereas PAEE/BW, step counts and moderate-intensity physical activity showed a negative correlation. PAL and PAEE adjusted for BW did not correlate with BMI (Fig. 1).

Energy expenditure and physical activity variables were also compared between the BMI quartiles. Age and height were not significantly different between the quartiles. TEE, step counts and moderate-intensity physical activity were significantly different between the quartiles. The fourth quartile

had significantly smaller PAEE/BW, step counts and moderate-intensity PAL compared with the other three quartiles. A statistically significant decrease in TEE between the BMI quartiles was still observed after adjustment for BW using ANCOVA (*P* = 0.026). However, PAEE did not show any significant difference between the BMI quartiles after adjustment for BW using ANCOVA (*P* = 0.155).

PAL, PAEE/BW and moderate-intensity physical activity negatively correlated with %BF (Table 2; Fig. 1). FM increased with %BF (*r* 0.876), whereas FFM did not (*r* 0.008). As shown in Fig. 1, after PAEE was adjusted for BW, a negative correlation was still observed between PAEE and %BF. A statistically significant difference was found between the %BF quartiles for PAL, PAEE/BW, step counts and moderate- and vigorous-intensity physical activities. A significant difference in TEE and PAEE was still found between the %BF quartiles after adjustment for BW using ANCOVA (*P* = 0.001 and 0.002). PAEE was lower in the second and fourth quartiles than in the first quartile when it was adjusted for BW using ANCOVA. PAL was lower in the second and fourth quartiles than in the first quartile, whereas there was no significant difference between the second and fourth quartiles.

Discussion

The present study aimed at comparing the relationship of BMI and %BF with various aspects of daily physical activity between Japanese adult men investigated in the present study and Japanese adult women that we have reported

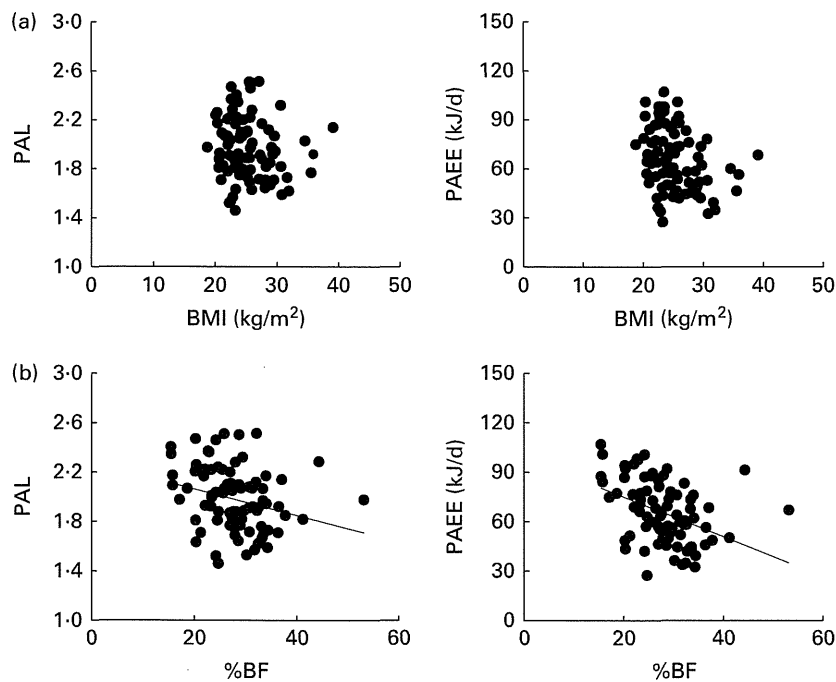


Fig. 1. Relationships between (a) BMI and physical activity level (PAL) or physical activity-related energy expenditure (PAEE) and (b) between body fat percentage (%BF) and PAL or PAEE. PAL = TEE/RMR, where TEE is the total energy expenditure; PAEE = 0.9 × TEE - RMR. %BF was negatively associated with PAL or PAEE, after adjustment for body weight, although this relationship was not observed between BMI and PAL or PAEE even after adjustment for body weight. (a) PAL: *r* -0.17; PAEE: partial *r* -0.05 (adjusted for body weight). (b) PAL: *r* -0.26, *P*<0.05; PAEE: partial *r* -0.27 (adjusted for body weight), *P*<0.05.

Table 2. Participants' characteristics, energy expenditure components and physical activity variables grouped according to body fat percentage (%BF)†
(Mean values and standard deviations)

%BF quartiles...	First (15.4–23.4)		Second (23.4–27.6)		Third (27.8–32.0)		Fourth (32.1–53.1)		P (ANOVA)	r [‡]
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Energy expenditure										
TEE (kJ/d)‖‖‖	12 166	1745	11 925	2137	11 687	1933	12 486	2288	0.632	0.123
RMR (kJ/d)	5736	687	6196	1081	5981	931	6707††§	988	0.009	0.329**
PAL	2.13	0.23	1.94†	0.26	1.97†	0.25	1.86††	0.23	0.005	-0.263*
PAEE (kJ/d)	5214	1234	4536	1383	4537	1275	4531	1445	0.245	-0.075
PAEE/BW (kJ/d per kg)††	80.7	17.9	64.3††	17.7	60.8††	15.4	54.7††	16.2	<0.001	-0.407**‖‖‖‖‖
Accelerometer										
Step counts (per d)	10 524	3067	9838	2876	9867	3181	7982†††§	2731	0.038	-0.202
Light, < 3 MET (min/d)	54.8	12.3	54.5	18.1	62.6	19.8	52.3	19.4	0.254	0.083
Moderate, 3 to < 6 MET (min/d)	39.9	14.7	37.8	19.2	34.5	16.8	25.1†††	14.8	0.024	-0.299**
Vigorous, 6 MET (min/d)	6.0	6.7	3.3	3.4	1.9	3.4	2.4	4.5	0.018	-0.138

TEE, total energy expenditure; PAL, physical activity level (TEE/RMR); PAEE, physical activity-related energy expenditure (0.9 × TEE – RMR); BW, body weight; MET, metabolic equivalents.

There was a significant correlation with BMI: * $P < 0.05$, ** $P < 0.01$.

Mean values were significantly different from those of the first quartile: † $P < 0.05$, †† $P < 0.01$.

Mean values were significantly different from those of the second quartile: ‡ $P < 0.05$.

Mean values were significantly different from those of the third quartile: § $P < 0.05$.

‖ Subjects were categorised by quartile. The number of subjects in each quartile was twenty-five. In the nine intensity levels (1–9) of physical activity assessed by an accelerometer, 1–3 indicates light intensity, 4–6 moderate intensity and 7–9 vigorous intensity.

†† The associations between BMI and physical activity variables and energy expenditure components, expressed as continuous variables, were examined by the linear regression analysis.

††† A statistical significance in TEE among the quartiles was still found even after adjustment for BW using ANCOVA ($P = 0.001$).

†††† A statistical significance in PAEE among the quartiles was still found even after adjustment for BW using ANCOVA ($P = 0.002$).

‖‖‖‖‖ A statistical significance between %BF and PAEE was still found even after controlling for BW (partial $r = 0.266$, $P = 0.015$).

previously⁽¹²⁾. The common finding in both studies was that BMI was negatively related to the number of steps and time spent in moderate-intensity physical activity, whereas %BF was negatively related to PAEE/BW and PAL.

No significant correlation was observed between BMI and PAL in Japanese adult men, a finding consistent with previous studies in Japanese women⁽¹²⁾ and Western populations^(9–11). On the other hand, Toozee *et al.*⁽¹⁹⁾ reported that normal (BMI < 25 kg/m²) and overweight (BMI 25 to < 30 kg/m²) women had a higher PAL compared with obese women (BMI ≥ 30 kg/m²). This relationship was not observed in adult men in their study.

However, grossly obese subjects often showed a lower PAL. Prentice *et al.*⁽⁹⁾ reported that grossly obese men (BMI ≥ 35 kg/m²) tended to have a lower PAL (mean 1.52) compared with normal-weight and obese men (BMI 18.5 to < 35 kg/m²) whose mean PAL values ranged between 1.80 and 1.86. In the present study, the number of grossly obese men was three and their PAL values were 1.77, 1.94 and 2.14, respectively. Therefore, the lower mean PAL value of 1.52 reported by Prentice *et al.*⁽⁹⁾ was not observed in grossly obese men in the present study. Similarly, Das *et al.*⁽¹¹⁾ reported that PAL did not differ in grossly obese women within the BMI range of 37.5–77 kg/m².

In contrast to the lack of the relationship between BMI and PAL, we showed that PAEE/BW was significantly lower in the highest quartile of BMI in Japanese adult men, a result similar to that observed in Japanese women⁽¹²⁾. This result is also in agreement with the study by Schoeller⁽²⁰⁾, which reported that obese Western populations had a lower PAEE/BW compared with non-obese populations. However, the present study revealed that there was no significant relationship between BMI and PAEE, following adjustment for BW as a covariate. Despite the convenience of correcting for body size, dividing PAEE by BW should be interpreted with caution, as this may be subject to artifacts due to problems with a zero intercept or the requirement of proportionality.

Although no significant relationship was observed between BMI and PAEE after correction for body size, %BF negatively correlated with both PAL and PAEE/BW. In particular, a significant relationship was still found between %BF and PAEE even after adjustment for BW as a covariate. The present study also showed a similar relationship between PAEE/BW and %BF to that observed in women⁽¹²⁾, Chinese adults⁽²¹⁾ and Western men⁽²²⁾. Therefore, it is not necessary to consider ethnic differences in this relationship. Moreover, when the data were analysed using the %BF quartiles (Table 2), PAEE/BW decreased from the second quartile in which BMI was < 25 kg/m². These results are similar to those observed in Japanese women⁽¹²⁾, and suggested that daily physical activity differed according to the level of fat deposition, even in the normal-weight adult population. However, it is also important to note that PAEE corrected for BW has the limitation of assuming that all physical activities are weight-dependent.

The present study showed the negative relationship between BMI and step counts or moderate-intensity physical activity, which is likely to that observed in Japanese women. However, Mitsui *et al.*⁽²³⁾ reported that there was a significant

relationship between step counts and BMI in Japanese adult women, but not in men. This discrepancy in the relationship between BMI and step counts in Japanese subjects may be attributable to lower BW, BMI and step counts in the study by Mitsui *et al.*⁽²³⁾. Thus, physical activity variables assessed by an accelerometer may be useful for understanding daily physical activity in obese adult populations in large epidemiological studies.

On the other hand, time spent in moderate-intensity physical activity was significantly related to %BF in men, although this relationship was not observed in women⁽¹²⁾. Conversely, the number of steps was related to %BF not in men but in women. The reason for this discrepancy between the sexes was not clear, although it is possible that %BF may be related to the total energy expended in daily physical activity rather than to the part composed of PAEE or PAL that included the number of steps and exercise intensity.

The present study is limited by the cross-sectional study design, which makes it difficult to clarify the causality between obesity and physical inactivity. Therefore, we cannot exclude the possibility of reverse causality that obesity may cause physical inactivity. For example, obese subjects fatigue after taking a few steps because they have to propel a larger mass; as a result, the lowered physical activity may cause obesity⁽²⁴⁾. In line with this, men in the fourth quartile, almost obese, may have had less volitional movement during physical activity due to their excess weight. The possibility of reversal causality is in agreement with the longitudinal study by Luke *et al.*⁽²⁵⁾ demonstrating that physical inactivity on the basis of energy expenditure does not predict weight change.

The present study also has the following limitations. First, FFM hydration was assumed to be equal in all participants at a value of 0.732⁽¹⁴⁾; therefore, some errors in estimating FM obtained from FFM may have resulted from differences in the levels of obesity and sex. Second, PAL in the study was higher than 1.75, the value reported in the general population of Eastern and Western countries^(21,26–28). The average daily number of steps of about 9564 observed in the participants of the present study was also higher than that reported for Japanese adult men in another study, who generally walk an average of 7893 steps/d⁽²⁹⁾. This indicates that the individuals included in the present study may have been more physically active than adult men in the general Japanese population. However, the ranges of PAL were similar between men and women (1.46–2.51 and 1.36–2.52, respectively)⁽¹²⁾, which were within the PAL of the general population⁽³⁰⁾.

In conclusion, the present cross-sectional study in Japanese adult men showed a negative relationship between BMI and the number of steps and time spent in moderate-intensity physical activity. It also showed that men with higher fat deposition were less active on the basis of PAEE adjusted for BW and PAL. These observations are very similar to those reported in our previous study in adult Japanese women⁽¹²⁾. These cross-sectional studies cannot prove causality, and that obesity causes physical inactivity may be the case. Despite these limitations, the present study did provide information regarding which physical activity variables are appropriate

for use in a longitudinal study. Additional research using a longitudinal study design is required to examine the cause–effect relationships between obesity and physical inactivity including factors of dietary intake.

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Prediction Models Discriminating between Nonlocomotive and Locomotive Activities in Children Using a Triaxial Accelerometer with a Gravity-removal Physical Activity Classification Algorithm

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Abstract

The aims of our study were to examine whether a gravity-removal physical activity classification algorithm (GRPACA) is applicable for discrimination between nonlocomotive and locomotive activities for various physical activities (PAs) of children and to prove that this approach improves the estimation accuracy of a prediction model for children using an accelerometer. Japanese children (42 boys and 26 girls) attending primary school were invited to participate in this study. We used a triaxial accelerometer with a sampling interval of 32 Hz and within a measurement range of ± 6 G. Participants were asked to perform 6 nonlocomotive and 5 locomotive activities. We measured raw synthetic acceleration with the triaxial accelerometer and monitored oxygen consumption and carbon dioxide production during each activity with the Douglas bag method. In addition, the resting metabolic rate (RMR) was measured with the subject sitting on a chair to calculate metabolic equivalents (METs). When the ratio of unfiltered synthetic acceleration (USA) and filtered synthetic acceleration (FSA) was 1.12, the rate of correct discrimination between nonlocomotive and locomotive activities was excellent, at 99.1% on average. As a result, a strong linear relationship was found for both nonlocomotive ($\text{METs} = 0.013 \times \text{synthetic acceleration} + 1.220$, $R^2 = 0.772$) and locomotive ($\text{METs} = 0.005 \times \text{synthetic acceleration} + 0.944$, $R^2 = 0.880$) activities, except for climbing down and up. The mean differences between the values predicted by our model and measured METs were -0.50 to 0.23 for moderate to vigorous intensity (>3.5 METs) PAs like running, ball throwing and washing the floor, which were regarded as unpredictable PAs. In addition, the difference was within 0.25 METs for sedentary to mild moderate PAs (<3.5 METs). Our specific calibration model that discriminates between nonlocomotive and locomotive activities for children can be useful to evaluate the sedentary to vigorous PAs intensity of both nonlocomotive and locomotive activities.

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Introduction

Much research has shown that there is a positive relationship between physical activity (PA) and both physical and mental health outcomes in children [1,2]. It is currently recommended that children should be engaged in moderate to vigorous intensity physical activity (MVPA) for at least 60 minutes each day [2,3]. Therefore, it is important to evaluate the exact PA intensity needed to improve and maintain an individual's physical and emotional health.

Estimation of PA in children is particularly difficult, since children show PA of varying intensity with short duration [4,5]. PA is generally estimated in units of activity energy expenditure or

time engaged in MVPA. To date, a variety of methods has been used to measure PA in children and adolescents. Although questionnaires and self-report activity diaries are effective methods in population-based research, they have the limitations of being less valid and reliable, particularly in children [6].

Accelerometers have recently come into wide use as monitors of PA. Accelerometers have the advantages of being objective, cost effective, and minimally invasive [7]. Previous studies proposed prediction models of metabolic equivalents (METs) for children with accelerometers [8–16]. These prediction models were based on the linear relationship between oxygen uptake and acceleration during several typical activities that reflect daily lifestyle activities of children. Typically, the selected activities are of low ($<$

3 METs), moderate (3–5.9 METs) and vigorous intensity (≥ 6 METs). The slope and intercept of a predictive model of locomotive activities, such as walking and running, are different from those of nonlocomotive activities, like playing games, cleaning, playing with blocks, tossing a ball, and aerobic dance [9–11,15]. Interestingly, Crouter et al. [17] proposed a new child-specific, two-regression model (2 RM), which is able to discriminate between locomotive activities, such as continuous walking or jogging, and nonlocomotive activities, including lifestyle activity, on the basis of the variability in the accelerometer count. The estimation accuracy of PA with the 2 RM depends on the sensitivity of discriminating between locomotive and nonlocomotive activities [18,19]. We also suggested a new calibration model that could discriminate locomotive activities from nonlocomotive activities in adults with a triaxial accelerometer based on the ratio of raw synthetic acceleration to filtered synthetic acceleration without gravity acceleration (gravity-removal physical activity classification algorithm [GRPACA]) [20,21]. The rate of correct discrimination between nonlocomotive (household) and locomotive activities was 98.7% for 11 selected activities in adults [21].

Our initial aim was to examine whether the GRPACA is able to discriminate between locomotive and nonlocomotive activities for various PAs of children. Our second aim was to prove that this discrimination method improves the estimation accuracy of the prediction model for children using an accelerometer.

Materials and Methods

Participants

Healthy Japanese children (42 boys: 15 who were 6–9 years of age, and 27 who were 10–12 years of age and 26 girls: 14 who were 6–9 years of age, and 12 who were 10–12 years of age) attending primary school were invited to participate in this study via a public advertisement. None of the participants had physical impairments that could affect daily life activity or took any medications that could affect metabolism. All participants and parents were fully informed of the purpose of the study, and written informed consent was obtained from parents on behalf of the participants prior to the start of the study. This study was conducted according to the guidelines of the Declaration of Helsinki, and all procedures involving human participants were approved by the Ethical Committee of the National Institute of Health and Nutrition.

Anthropometry

Body weight was measured to the nearest 0.1 kg with a digital balance (YL-65S, YAGAMI Inc., Nagoya, Japan), and height was measured on a stadiometer to the nearest 0.1 cm (YK-150D, YAGAMI Inc., Nagoya, Japan). Body mass index (kg/m^2) was calculated as body weight divided by the square of body height.

Procedures

To avoid diet-induced thermogenesis, the children visited the laboratory in the morning, three hours after breakfast. After the study protocol was fully explained, anthropometric measurements were taken. Next, participants were asked to rest for 30 minutes, and then the resting (in the seated position on a chair) metabolic rate was measured for 7 minutes; in children, it was measured while the child was viewing a video (e.g. Disney movie) to avoid fidgeting [22]. In addition, we asked the participants to put their hands on their thighs and to keep their feet on the floor during the measurement. Next, the children performed 11 PA for approximately 3 to 7 minutes, in addition to 3 minutes to obtain steady state (Table 1). First, nonlocomotive activities excluding throwing

a ball were performed in order of PA intensity (lower to higher) with a few minutes of recovery between tasks, and after an approximately 10-minute break, climbing down and up activities were performed sequentially. Next, participants performed the throwing a ball activity. Locomotive activities were also conducted in order of PA intensity (lower to higher) with a few minutes of recovery between activities. All participants wore a triaxial accelerometer on the waist, tightly attached with a belt, during each activity. Before the experiment started, the accelerometers were synchronized using a wave clock for reference. Measurement of each activity began after a preliminary period that was needed to reach a steady-state condition with 3 minutes, based on our pilot study and previous studies [17,20,23]. The steady-state durations for climbing down and up were 2 minutes, because participants were moving to the implementation site on foot within a few minutes of the measurement of climbing down, and climbing up was performed after climbing down for 3 minutes. The energy expenditure (EE) of each activity was calculated from oxygen consumption (VO_2) and carbon dioxide production (VCO_2) with Weir's equation [24]. To calculate the METs, we divided the EE during each activity by the measured value of the metabolic rate of the participant when seated on a chair.

Triaxial Accelerometer

We used a triaxial accelerometer with 4 GB of memory (Omron Healthcare, Kyoto, Japan) consisting of a Micro electro-mechanical system-based accelerometer (LIS3LV02DQ; ST-Microelectronics), which responds to both acceleration due to movement and gravitational acceleration. The device for children measured 74 mm \times 46 mm \times 34 mm and weighed 60 g, including batteries. It was designed to detect accelerations in the vertical (x), anteroposterior (y), and mediolateral (z) axes with each activity at a rate of 32 Hz to 12-bit accuracy. The acceleration obtained from these specifications was passed through a high-pass filter with a cut-off of 0.7 Hz to exclude gravitational acceleration. We calculated the integral of the absolute value of the accelerometer value (synthetic acceleration), the square root of the sum of the square of the absolute acceleration from three axes (synthetic acceleration = $(X^2 + Y^2 + Z^2)^{0.5}$). Finally, this device could record the synthetic acceleration of a 10-s epoch length within a measurement range of ± 6 G and with a resolution of 3 mG. We analysed the acceleration data converted into a 10-s epoch length when collecting the expired gas for each activity. The reliability of this device was validated by the manufacturer, and is reported in technical reports (unpublished). The reliability test referred to the procedures of Japanese Industrial Standards (JIS7200:1993), according to which a pedometer is validated with a vibration exciter.

Indirect Calorimetry

Respiratory gas samples were analysed with the Douglas bag method, in which each participant was fitted with a facemask (No.09759, YAGAMI Inc., Nagoya, Japan) and breathed into a Douglas bag (No.35060, YAGAMI Inc., Nagoya, Japan). Participants performed calibration tasks person-to-person with an assistant who was holding the 50 L or 100 L-sized Douglas bag. The assistant opened a cock of the Douglas bag to collect the expired gas at the same time as the steady-state period finished, and then closed it when measurement finished without hindrance. The bag concentrations of oxygen and carbon dioxide were analyzed by a mass spectrometer (ARCO-1000; Arco System Inc., Kashiwa, Japan) that has recently come into wide use in several countries, in particular, Japan [20,25]. The precision of the expired gas measurement was 0.02% for oxygen and 0.06% for

Table 1. Description of performed calibration tasks.

Tasks	Content of activity	Intensity	Steady state (min)	Gathering expired gas (min)*
Nonlocomotive				
desk work	handwriting letters at a desk	light	3.0	4.0
Nintendo DS	playing Nintendo DS with sitting on the floor	light	3.0	3.0
sweeping up	sweeping floor (about 17 m ²) while moving	light	3.0	3.0
clearing away	placing books from floor onto a bookshelf	light	3.0	3.0
washing the floor	wiping down the floor with a cloth in a squatting position	moderate	3.0	2.0
throwing a ball	playing catch with a large ball with a partner	moderate	3.0	3.0
Locomotive				
climbing down	climbing down stairs according to a pace leader	moderate	2.0	1.0
climbing up	climbing up stairs according to a pace leader	vigorous	2.0	1.0
normal walking	normal walking speed according to a pace leader (60 m/min) on the ground	moderate	3.0	2.0
brisk walking	brisk walking speed according to a pace leader (80 m/min) on the ground	moderate	3.0	2.0
Jogging	jogging according to a pace leader (early grades: 100 m/min, late grades: 120 m/min)	vigorous	3.0	2.0

*We collected expired gas for 1 to 4 min after steady state for 2 or 3 min.
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carbon dioxide. The expired gas volume was measured with a certified dry gas meter (DC-5; Shinagawa Co., Ltd., Tokyo, Japan), the accuracy and precision of which were maintained within 1% of the coefficient of variation.

Selection of Physical Activity for Calibration Models

We gathered information about the children's habitual PA behavior at school and after school from direct interviews of another group of children and public reports of an education committee. Based on those sources of information, we selected 11 PAs for children that consisted of sedentary and light (< 3 METs), moderate (3–5.9 METs), and vigorous activity (≥ 6 METs), according to the compendium of PAs [26,27], to produce a calibration model.

Discriminative Method

In our previous study, we reported an algorithm for the classification of nonlocomotive (household) and locomotive activities based on the ratio (e.g. cut-off value for adults, 1.16) of unfiltered synthetic acceleration (USA) to filtered synthetic acceleration (FSA) [21]. FSA was defined as the integrated acceleration ($(X^2 + Y^2 + Z^2)^{0.5}$) after the gravitational acceleration was removed from each dimensional acceleration (X, Y, Z) by passing through a second-order Butterworth high-pass filter [21]. Thus, the most important difference between USA and FSA is that FSA is not affected by a change in gravitational acceleration, while USA is. In adults, the rate of correct discrimination of nonlocomotive (e.g. household) from locomotive activities was

98.7% for 11 selected activities with the ratio (USA/FSA) [21]. Therefore, in this study, this discriminative procedure was applied to the children's calibration model, and we aimed to determine a cut-off value for children.

Statistical Analysis

Statistical analysis was performed with JMP version 8.0 for Windows (SAS Institute, Tokyo, Japan). All results are shown as mean ± standard deviation (SD). In the present study, we carried out multiple regression analysis with a stepwise method to examine the effects of weight, age and sex, and then analysis of covariance (ANCOVA) to assess the interaction (age × sex) on the measured METs prior to statistical analyses. The determination coefficient (R^2) was used to evaluate the relationships between variables. One-way analysis of variance (ANOVA) was used to compare measured METs with predicted METs. Mean differences and limits of agreement between predicted METs and measured METs were determined by Bland and Altman plots [28]. Receiver-operating characteristic (ROC) curve analysis was applied to the acceleration data to assess the cut-off value for classification of nonlocomotive and locomotive activities. $P < 0.05$ was considered statistically significant.

Results

First, we divided the children into two groups: a development group and a cross-validation group. We randomly selected participants stratified by sex and age (6–9 yrs and 10–12 yrs).

Table 2. Physical characteristics of the participants.

	Development group				Cross-validation group				Total participants			
	Boys (30)		Girls (18)		Boys (12)		Girls (8)		Boys (42)		Girls (26)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (yrs)	10.0	1.8	9.2	2.1	10.1	1.5	8.8	1.2	10.0	1.7	9.0	1.8
Height (cm)	140.2	12.4	134.9	14.2	141.1	7.5	131.4	10.6	140.5	11.5	134.4	12.6
Weight (kg)	34.0	11.0	30.2	9.2	33.7	5.2	27.2	6.3	33.9	9.9	29.8	8.2
BMI (kg/m ²)	16.9	2.9	16.2	2.2	16.8	1.6	15.6	1.6	16.8	2.6	16.1	2.1

BMI: body mass index, SD: standard deviation.
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Characteristics of all children, the development group and the cross-validation group are shown in Table 2.

Discrimination with the ratio of USA/FSA provided the highest rate of correct discrimination, 99.8%, when the value of the ratio was 1.12 (Figure 1, Table 3). Therefore, we calculated the estimated METs through standard equations according to the results of discrimination with the ratio of 1.12, and then compared these values with the measured METs. The relationship between synthetic acceleration and METs is shown in Figure 2 (development group: n = 48). Plots of nonlocomotive activities were different from those of locomotive activities. In addition, plots of climbing down and up were located above and below the line, respectively. The linear regression equation is as follows:

Nonlocomotive Activities Equation (Development Group: n = 48)

$$\text{METs} = 0.013 \times \text{synthetic acceleration} + 1.235, \quad R^2 = 0.752, \quad \text{RSME} = 0.694 \text{ (standard equation)}$$

Locomotive Activities Equation (Development Group: n = 48)

$$\text{METs} = 0.005 \times \text{synthetic acceleration} + 0.878, \quad R^2 = 0.884, \quad \text{RMSE} = 0.651 \text{ (standard equation)}$$

Next, we examined the cross-validation of the new calibration model in the cross-validation group (n = 20). The rate of correct discrimination was 99.1% when the cut-off value of 1.12 was used to discriminate PAs in cross-validation group. The absolute differences were less than or equal to 0.50 METs, excluding climbing down and up (Table 4). Finally, we proposed an equation from the data of all participants (the development group combined with the cross-validation group).

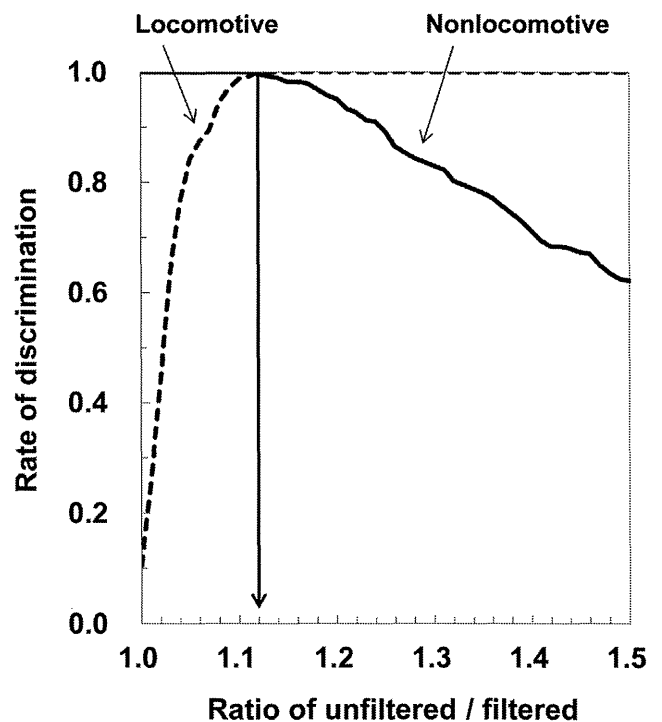


Figure 1. Probability of correctly detecting locomotive and nonlocomotive activities in the development group (n = 48).
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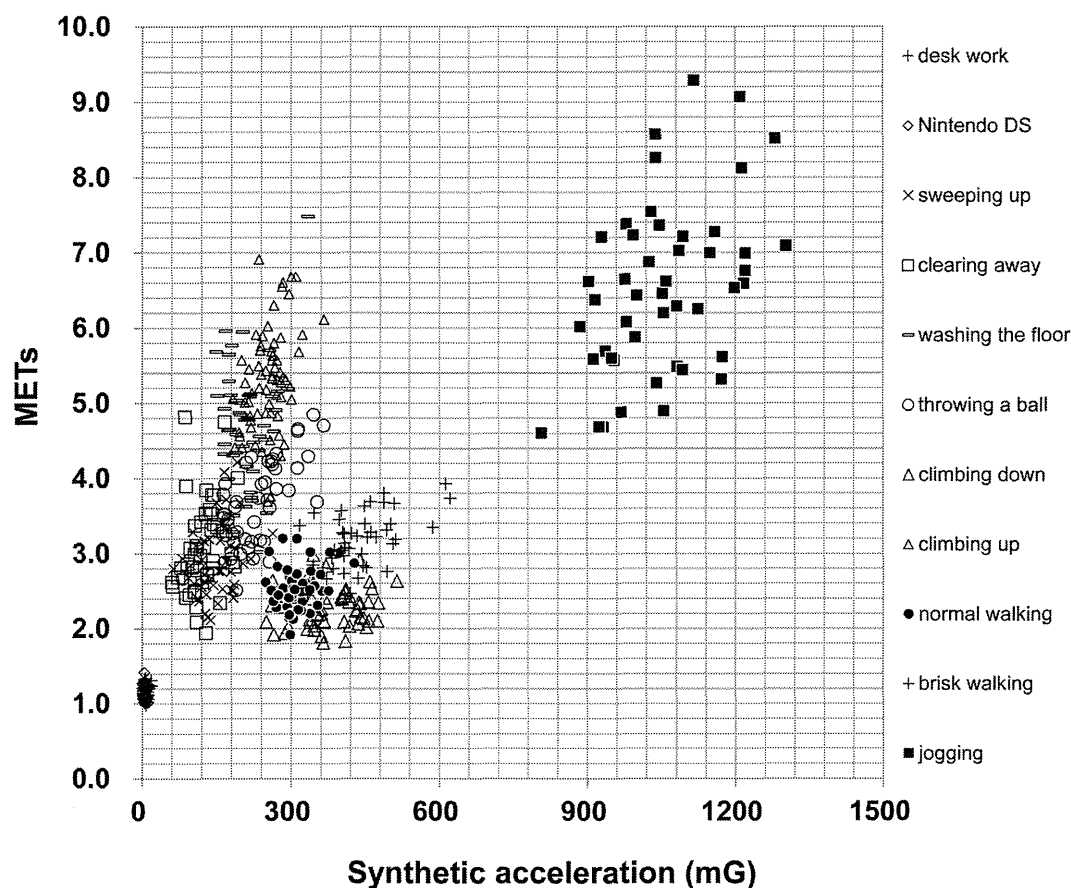


Figure 2. Relationship of synthetic acceleration to measured METs in nonlocomotive and locomotive activities in the development group (n = 48).

doi:10.1371/journal.pone.0094940.g002

Nonlocomotive Activities Equation (Total Participants: n = 68)

$\text{METs} = 0.013 \times \text{synthetic acceleration} + 1.220$, $R^2 = 0.772$, $\text{RMSE} = 0.664$ (standard equation)

Locomotive Activities Equation (Total Participants: n = 68)

$\text{METs} = 0.005 \times \text{synthetic acceleration} + 0.944$, $R^2 = 0.880$, $\text{RMSE} = 0.639$ (standard equation)

Furthermore, the inclusion of weight, chronological age and sex significantly improved the prediction accuracy of the locomotive equation. Chronological age and sex were significant variables in the nonlocomotive equation. However, the interaction term between chronological age and sex was not significant in either equation (Table 5).

We compared each MET value obtained from the standard equation and the multiple regression equation with the METs measured during each PA (Table 6). Although a slight improvement in the predictive equation (R^2 and RMSE) was observed in both nonlocomotive and locomotive activities, we could not find significant improvements in the predictive ability for each activity (Table 6).

The predicted values from standard equation for washing the floor (-0.65 ± 0.88 ; METs, $-11.4 \pm 18.8\%$) and climbing up (-2.91 ± 0.74 ; METs, $-54.2 \pm 9.1\%$) were significantly underestimated compared with the measured values. The predicted values of desk work (0.17 ± 0.11 ; METs, $15.7 \pm 11.2\%$), Nintendo DS (0.18 ± 0.10 , METs, $17.1 \pm 10.4\%$), throwing a ball (0.53 ± 0.60 ,

METs, $15.7 \pm 18.1\%$) and climbing down (0.67 ± 0.42 ; METs, $30.9 \pm 20.2\%$) were significantly overestimated. However, we did not observe significant differences between the predicted values and the measured values for sweeping up, clearing away, or brisk walking and jogging (Table 6).

In addition, the differences between the measured METs and the predicted METs from each equation were determined by Bland-Altman analysis (Figure 3). The standard equation showed a mean difference of -0.13 METs and limits of agreement (± 2 SD) from $+2.06$ to -2.33 METs. The multiple regression equation showed a mean difference of -0.17 METs and limits of agreement (± 2 SD) from $+1.91$ to -2.26 METs.

Discussion

Many studies have reported that accelerometers are excellent devices for the estimation of locomotive activities, such as walking and jogging on a treadmill or on the ground [29,30]. However, recently, several studies reported that it was difficult to estimate PA intensity for children using the existing predictive model [10,11,14–17], because the habitual PA behaviors of children are more complex and poorer economically [31,32], and they change more frequently than those of adults [4,5]. To be precise, a predictive equation based on locomotive activities led to an underestimation of PA intensity during nonlocomotive activities, such as household tasks [11]. This might mean that discriminating locomotive from nonlocomotive activities contributes to the estimation accuracy of PA intensity in children. Therefore, in

Table 3. Rate of correct discrimination of nonlocomotive from locomotive activities.

Threshold	1.12*		1.13		1.14		1.15		1.16#	
	Development group (48)	Cross-validation group (20)	Development group (48)	Cross-validation group (20)	Development group (48)	Cross-validation group (20)	Development group (48)	Cross-validation group (20)	Development group (48)	Cross-validation group (20)
<i>Nonlocomotive</i>										
desk work	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Nintendo DS	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
sweeping up	100.0	100.0	97.9	100.0	97.9	95.0	95.8	95.0	95.8	95.0
clearing away	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
washing the floor	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
throwing a ball	97.9	100.0	97.9	95.0	95.8	95.0	93.8	90.0	93.8	90.0
<i>Locomotive</i>										
climbing down	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
climbing up	100.0	90.0	100.0	95.0	100.0	95.0	100.0	100.0	100.0	100.0
normal walking	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
brisk walking	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
jogging	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Total discrimination	99.8%	99.1%	99.6%	99.1%	99.4%	98.6%	99.1%	98.6%	98.9%	98.6%

*shows the excellent cut-off value of children to discriminate between locomotive and nonlocomotive activity in this study.

#shows the cut-off value of adults to discriminate between locomotive and nonlocomotive activity which was proposed in our previous study [20].

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Table 4. Absolute and percentage differences between measured and predicted METs from each equation model for nonlocomotive and locomotive activities in the cross-validation group (n=20).

	Predicted METs		Measured METs		Absolute difference		% difference		P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Nonlocomotive									
desk work	1.34	0.06	1.15	0.13	0.19	0.13	17.5	15.0	<0.01
Nintendo DS	1.30	0.03	1.11	0.09	0.18	0.10	17.9	9.1	<0.01
sweeping up	3.29	0.72	3.15	0.73	0.14	0.46	5.8	14.6	NS
clearing away	2.77	0.40	3.01	0.58	-0.25	0.42	-6.5	12.8	NS
washing the floor	3.91	0.40	4.41	0.69	-0.50	0.79	-9.0	18.9	<0.01
throwing a ball	4.26	0.78	3.76	0.82	0.48	0.45	14.9	13.4	<0.05
Locomotive									
climbing down	2.88	0.27	2.26	0.28	0.58	0.41	29.1	20.5	<0.01
climbing up	2.20	0.20	5.28	0.69	-3.08	0.61	-58.0	4.7	<0.01
normal walking	2.54	0.21	2.58	0.24	-0.04	0.36	-0.6	13.8	NS
brisk walking	3.21	0.25	3.16	0.25	0.05	0.36	2.1	11.3	NS
Jogging	6.44	0.48	6.20	0.77	0.23	0.83	5.2	14.7	NS

P<0.05 and <0.01 show that mean values were significantly different compared with measured METs. METs; metabolic equivalents, SD; standard deviation, NS; not significant.
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Table 5. Effect of weight, age and sex on predictive ability by multiple regression analysis.

Independent variable	Intercept	Regression coefficient	P value	Adjusted R ²	RMSE
Nonlocomotive					
Model 1					
synthetic acceleration (mg)	1.220	0.013	<0.001	0.772	0.664
Model 2					
synthetic acceleration (mg)	-0.537	0.013	<0.001	0.816	0.596
weight			NS		
age		0.170	<0.001		
sex (boys:0, girls:1)		0.076	<0.05		
Locomotive					
Model 1					
synthetic acceleration (mg)	0.944	0.005	<0.001	0.880	0.639
Model 2					
synthetic acceleration (mg)	-0.925	0.005	<0.001	0.925	0.508
weight		0.032	<0.001		
age		0.085	<0.01		
sex (boys:0, girls:1)		0.092	<0.05		

RMSE; root mean square error, NS; not significant.
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the current study, we tried to examine whether the GRPACA, which was developed in our calibration model for adults, is able to discriminate various PAs in children, and to prove that this discrimination method improves the estimation accuracy of the prediction model for children using an accelerometer.

Our first key finding was that it might be possible to apply the discrimination procedures developed in adults to any participant with various activity components and patterns. In our previous study, we found that the percentage of correct discrimination with the GRPACA in adults was remarkable, 98.7%, when the ratio of USA/FSA was 1.16 [21]. In the present study, when the threshold of discrimination, which was similar to that in the previous study, was 1.12, the rate of correct discrimination was excellent, at 99.1% on average (Figure 1, Table 3). As the discrimination method that used the coefficient of variation in a previous study was 97% for locomotive activities and 89.5% for nonlocomotive activities [17], our discrimination procedure had a better rate of correct discrimination. It follows that our specific calibration model could evaluate the PA intensity of children with an estimation accuracy of a mean difference of -0.13 METs and limits of agreement (± 2 SD) from +2.06 to -2.33 METs, similar to the success we obtained with the adult model in our previous study for adults [20,21]. This finding was supported by a strong linear relationship in the two prediction formulas and a cross-validation trial with another group of children (Table 4). These results suggested that our specific model, established according to the procedure of the adult model, was well suited to evaluate the PA of children.

We did not simultaneously compare our device with major devices, such as ActiGraph. However, our calibration procedures followed the procedures used in several calibration studies [11–17], which enabled comparison of the results in the present study with previous studies that used a common device. For example, a proposed single equation using a common device such as ActiGraph, Actical or RT3 provides average prediction errors of more than about 20% for nonlocomotive activities, calculated from average published values like VO_2 ($\text{ml}/\text{kg}^{0.75}/\text{min}$), activity energy expenditure ($\text{kcal}/\text{kg}/\text{min}$) and METs [14,33,34,35].

Moreover, when our model was compared with the 2 RM with ActiGraph proposed recently, the differences between the predicted METs and the measured METs in the current study were slightly smaller than those of the previous study [17]. To be more precise, the differences with ActiGraph for vigorous intensity PAs, such as sportwall and running, were -1.8 to METs and -1.1 METs [17], respectively, while the differences with our model were 0.23 METs for similar-intensity PAs like jogging. Furthermore, the difference with our model, which was within 0.50 METs for all PAs including sedentary to vigorous intensities, except for climbing up and down, was slightly smaller than in the previous study (within 0.6 METs) [17]. Actually, another study also indicated that the 2 RM with ActiGraph had a disadvantage for sedentary and high intensity PAs [36]. In the current study, although there were significant differences between the measured METs and the predicted values from standard equations in washing the floor, throwing a ball, and climbing down and climbing up, mean differences compared to the measured METs in overall activities were small (-0.13 ± 1.09 METs). Mean differences between the predicted METs and the measured METs only in sedentary behaviors to light intensity PAs (<3.0 METs), which consumed the highest percentage of time per day [37], were still minimal (-0.20 ± 0.33 METs) in the current study.

The finding that our procedure could lead to comparable estimation accuracy in both nonlocomotive and locomotive activities was also significant. The cause might depend on the fact that our model could assess upper-body activities such as sweeping up, clearing away, and throwing a ball accurately. Oshima et al. [21] indicated that when the acceleration sensor was attached to the waist of the individual, the USA/FSA ratio reflected dynamic changes in body posture. The waist is not in the upper body, but the inclination of the upper body accompanies that of the waist in most instances. Therefore, the gravitational acceleration signal at the waist reflects postural changes of the upper body during nonlocomotive activities, like household activities, to some degree.