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Abstract:

INTRODUCTION: Sedentary Behavior (SB) research has relied on accelerometer thresholds to distinguish between sitting/lying time (SLT) and light intensity physical activity (LIPA). Such methods may misclassify SLT, standing time (StT) and LIPA. This study examines the association between inclinometer-determined SB, physical activity (PA) and adiposity in an adolescent female sample. **METHODS:** Female adolescents (n = 195; mean age = 15.7 (SD = 0.9) yrs.) had body mass index (BMI) (median = 21.7 (Interquartile Range (IQR) = 5.2) kg/m²) and 4-site Σ skinfolds (median 62.0 mm; IQR 37.1) measured and wore an activPALTM activity monitor for 7 days. SLT, StT, breaks in SLT and bouts of SLT <30 and \geq 30 minutes in duration were determined from activPAL outputs. A threshold of 2997 counts/15s determined moderateto-vigorous PA (MVPA). All remaining time was quantified as LIPA. Mixed linear regression models examined associations between PA variables, SB variables and adiposity. RESULTS: Participants spent a mean of 65.3% (SD 7.1) of the waking day in SLT, 23.0% (SD 5.3) in StT, 5.6% (SD 1.5) in LIPA and 6.1% (SD 2.4) in MVPA. Significant effects for the percentage of LIPA (which excluded StT) with both BMI ($\beta = -4.38$: p = 0.0006) and Σ skinfolds ($\beta = -4.05$: p = 0.006) were identified. Significant effects for breaks in SLT with BMI (β = -0.30: p = 0.04) were also observed. No additional significant associations were found between activity measures and adiposity. CONCLUSION: Increased LIPA (excluding StT) and breaks in SLT were negatively associated with adiposity in this sample, independent of age. Interventional work should examine whether reducing SLT through breaks and increasing LIPA may prevent increases in adiposity in adolescent females.

Keywords: Sedentary, Light Physical Activity, activPAL, Obesity.

Introduction:

A large and consistent body of evidence has shown that increased levels of physical activity protects children and adolescents from the development of overweight and obesity (9), and that moderate-to-vigorous physical activity (MVPA) is an independent predictor of adiposity in children and adolescents (24, 27). To date, the majority of physical activity research has focused on MVPA (22). However, MVPA accounts for a very small proportion of total daily physical activity in youth, with data from US NHANES 2003-2004 identifying that objectively measured MVPA accounted for approximately 25 min/day for 12-15 year old females and 20 min/day for 15-19 year old females (29). It is now clear that the amount of energy expended through volitional exercise (e.g. MVPA) is not the dominant determinant of variability in daily energy expenditure in youth (12, 13).

Non-exercise activity thermogenesis (NEAT) is described as the energy expended throughout activities of daily living, and is composed of sitting/lying time (SLT), standing time (StT) and all light intensity physical activity (LIPA) (15). There is increasing interest in the association between NEAT and indices of health in epidemiological studies. It has been hypothesised that components of NEAT have opposite associations with health outcomes, whereby SLT may be negatively associated with health outcomes, while StT and LIPA may be beneficial to health outcomes. It is through these activities at the lower end of the activity intensity spectrum that the majority of total daily energy is expended (15). However, it is not clear which components of NEAT are associated with health indices, primarily due to limitations with existing measurement methodologies. The examination of the individual components of NEAT is extremely difficult due to the ubiquitous nature of such activities. The majority of research has employed self-report

measures to estimate time spent sedentary and in LIPA. Such measures have significant limitations due to recall difficulties and the use of surrogate measures of sedentariness, such as television viewing time (1, 16, 22, 25). More recent studies have employed accelerometer-based activity monitors as a measure of sedentary time and LIPA (1, 7, 18, 22). Although such devices have greater reliability and validity than self-report (16, 21), these measures rely on the lack of ambulation rather than postural position to estimate SLT. Consequently, this method of examining sedentary time often results in misclassification of StT and LIPA (6, 23). This is a significant limitation, as the behavior of sedentariness is defined as any "waking behavior spent in a sitting or reclining position that require an energy expenditure of <1.5 metabolic equivalents" (28), and consequently would not include StT.

Relationships between objectively measured SLT, StT, LIPA and indices of health in young people are poorly understood. Evidence in child and adolescent samples have found that associations between sedentary time and indices of health do not persist when controlling for MVPA (2, 20), while no literature is currently available on the associations between objectively measured StT and indices of health. Similarly, limited information is available on the associations between LIPA and indices of health in young people, while no research has distinguished SLT from StT or StT from LIPA to provide a more comprehensive measure of both SLT and LIPA in any population.

The purpose of this study was to examine the associations between SLT, StT and LIPA and adiposity in a sample of adolescent females using an inclinometry-based activity monitor.

Methods and Procedures:

Cross-sectional data was collected from a convenience sample of 7 urban and 6 rural secondary schools in the mid-western region of Ireland between 2009 and 2011. Participants were randomly selected from a list of all 13-18 year old female students enrolled in each school. To be eligible for inclusion in this study, participants were required to have no injuries or illnesses which negatively impacted on their participation in physical activity. The numbers recruited from each school varied by school size. This study was reviewed and approved by the University of Limerick research ethics committee. A total of 216 students provided written informed participant and parental consent and participated in the full test days. Due to insufficient activity monitor data, 21 datasets were excluded from analysis. A total of 195 valid datasets were included in the present analysis.

Measurement of Physical Activity and Sedentary Behaviors:

The inclinometer-based activity monitor employed in this research was the activPALTM Professional Physical Activity Monitor (PAL Technologies Ltd., Glasgow, UK). A detailed description of the characteristics of the activPALTM has been described elsewhere (3). Briefly, the activPAL is a single unit uniaxial accelerometer measuring 53 x 35 x 7 mm and weighing approximately 15 grams. The device was worn on the midpoint of the anterior aspect of the thigh, and was attached to the skin using a hydro-gel adhesive pad (PAL*stickie*). For consistency, participants were instructed to wear the device on their right thigh only for a 7 day period. The device was worn for 24 hrs./day throughout the measurement period, and was only removed for bathing or for water-based activities. Proprietary algorithms classified the individual's free-living activities into SLT, StT, stepping time, step count and activity counts. The activPAL

communicates with a Windows (Microsoft Corporation, Microsoft Excel 2010, One Microsoft Way, Redmond, WA, USA) compatible PC using a USB interface.

Measurement of Adiposity:

Height was measured to the nearest 0.25 cm using a portable wall stadiometer (Seca model 214, Seca Ltd., Birmingham, UK). Body weight was measured to the nearest 0.01 kg using a portable electronic scale (Seca model 77, Seca Ltd., Birmingham, UK). Body mass index (BMI) was calculated by dividing weight (kg) by height (m)² and BMI percentiles were calculated based on age and sex in accordance with Centre for Disease Control and Prevention (CDC) reference data (11). Skinfold measurements were obtained from 4 sites (bicep, triceps, subscapular and iliac crest) according to the skinfold protocol of the International Society for the Advancement of Kinanthropometry (17). Skinfold thickness was measured to the nearest 0.25 cm using a Harpenden skinfold calliper (Cranlea & Co, Birmingham, UK). All anthropometric measures were obtained during a single visit to each school. Three trained investigators carried out the anthropometric measures. Inter-tester technical error of measurement was set at 5% for skinfold thickness measures. If technical error of measurement was set at 5% for skinfold thickness taken and the median value was used for analysis.

Data Processing:

A 7 day measurement protocol which provides a minimum of 4 valid days of activity data (including one weekend day) has been suggested as a valid recording duration for adolescent populations (30). For the purpose of this analysis, a valid day was classified as a measured day

with \leq 4 hours non-wear time during waking hours. Non-wear time was defined as a period with \geq 60 minutes of consecutive zero activity counts. This method for identifying periods of non-wear time is consistent with free-living data reduction methodologies (8). The non-wear periods for each day were summed, and all measurement days with \geq 4 hours of non-wear time during waking hours were removed. Participants that did not provide 4 valid days of activity monitoring data (including at least 1 weekend day) were removed from all further analysis (n = 21). For all remaining participants, the daily non-wear time was summed, and the measured waking day was adjusted accordingly.

All SB and PA variables were presented as a percentage of waking time. To estimate bed hours, the first registered non-sedentary epoch after 7 am was identified as rise time. This time was chosen as manual pre-screening of participants rise times identified no participants woke prior to 7 am. The last registered non-sedentary epoch which was followed by an uninterrupted sedentary period (>2 hours) was identified as the time participants went to bed. The amount of waking time was then calculated as Waking Hours = Bed time – Rise time.

Free-Living Physical Activity and Sedentary Behaviors:

The activPAL was used to estimate daily physical activity and sedentary behavior variables, including SLT, breaks in SLT, SLT bouts of <30 minutes in duration, SLT bouts of \geq 30 minutes in duration, StT, LIPA including StT, LIPA excluding StT, and MVPA. A detailed description of the methodologies used to examine these physical activity and sedentary behavior variables have previously been reported (3). SLT was defined as all time spent in a sitting or lying posture, and was calculated by summing the total number of seconds spent in sitting/lying postures over the waking measurement period. Breaks in SLT were defined as any transition from a sitting/lying

posture to a standing posture, and breaks in SLT were summed over the waking measurement period. A SLT bout of <30 minutes in duration is defined as the amount of time spent in a sitting/lying posture for a duration of less than 30 minutes, while a SLT bout of \geq 30 minutes in duration is defined as the amount of time spent in a sitting/lying posture for a duration of greater than 30 minutes. The amount of time spent in SLT bouts of <30 minutes in duration and ≥ 30 minutes in duration were summed over the waking measurement period. StT was defined as all time spent in a standing position where no locomotion/stepping was achieved (e.g. standing still), LIPA was defined as all time spent in a locomotive/stepping behavior which was at an intensity of less than 3 metabolic equivalents (METS) (e.g. slow walking, household chores etc.), while MVPA was defined as all time spent in a locomotive/stepping behavior which was at an intensity of greater than 3 METS. For MVPA, a threshold of 2997 counts/epoch (15 sec⁻¹) was used to estimate metabolic equivalents for each 15 second period, where MVPA was defined as ≥ 3 metabolic equivalents (4). LIPA excluding StT (excl. StT) was then calculated as: LIPA (excl. StT) = [24 hours - (SLT + StT+ MVPA)]. LIPA including StT (incl. StT) was calculated as LIPA (incl. StT) = StT + LIPA (excl. StT). SLT was adjusted by subtracting non-wear time from SLT. This method of examining non-wear time data was completed as 1) no records for the types of activity completed during non-wear time were collected and 2) non-wear time would otherwise be categorised as SLT. Total daily wear time was calculated by subtracting non-wear time from the waking measurement period, and each variable was then divided by the total daily waking wear time to derive the percentage of waking time spent in each physical activity and sedentary behavior variable.

Statistical Analysis:

Descriptive statistics were calculated, and are presented as mean (SD) for normally distributed variables or median (IQR) for skewed distributions. Spearman's correlation coefficient (r_s) was used to measure the association between physical activity and sedentary behavior variables and both BMI and Σ skinfolds. Mixed linear regression models were used to examine these relationships after adjusting for age and the clustering of participants within schools. School was included as a random effect in the models and age was included as a fixed effect. Separate models were fitted for each physical activity and sedentary behavior variable as a predictor of outcomes (BMI and Σ skinfolds). Models which adjusted for MVPA were also fitted, after testing for collinearity of MVPA with the other physical activity and sedentary behavior variables. Residual analysis was used to check assumptions underlying the model and model fit was assessed using Akaike's Information Criterion (AIC) and Schwarz's Bayesian Information Criterion (BIC). A 5% level of significance was used for all statistical tests. Statistical analyses were undertaken using IBM SPSS Statistics v. 20 (Armonk, New York, USA) and SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results:

Descriptive statistics for the sample are presented in Table 1. A broad BMI range was observed $(15.4-41.3 \text{ kg/m}^2)$. A total of 9 participants (4.6%) were classified as underweight, 132 participants (67.7%) were classified as having a normal weight, 41 participants (21.0%) were classified as overweight and 13 participants (6.7%) were classified as obese. A total of 29 participants provided 4 valid days of accelerometer data (14.9%), with 140 participants providing 5 valid days (71.8%) and 26 participants providing 6 valid days (13.3%). Of all participants

included in this analysis, a total of 180 provided data on both weekend days (92.3%), with 15 participants providing data on one weekend day (7.7%). The average percentage of waking time spent in each activity variable was 65.3% for SLT, 23.0% for StT, 5.6% for LIPA and 6.1% for MVPA. Of the daily waking hours, an average of 9.6 hrs. (SD = 1.2) was spent in SLT, 3.4 hrs. (SD = 0.8) in StT, 0.8 hrs. (SD = 0.2) in LIPA and 0.9 hrs. (SD = 0.4) in MVPA. When examined together, LIPA (incl. StT) accounted for 28.7%, or 1.7 hrs. (SD = 0.5), of the waking measurement period.

Of all physical activity and sedentary behavior variables examined, the percentage of waking time spent in LIPA (excl. StT) had the strongest association with BMI percentile and Σ skinfolds (Table 2). A weak to moderate negative association was found between increasing LIPA (excl. StT) and both BMI percentile ($r_s = -0.24$, p < 0.001) and Σ skinfolds ($r_s = -0.25$, p < 0.001).

The association between percentage of waking time spent in LIPA (excl. StT) and both BMI percentile ($\beta = -4.38$: p = 0.0006) and Σ skinfolds ($\beta = -4.05$: p = 0.006) was significant, after adjustment for age and the clustering of participants in schools (Table 3). The negative parameter estimate for β suggests an inverse relationship between increasing LIPA (excl. StT) and excess adiposity. A significant association was also observed between the number of breaks in SLT and BMI percentiles ($\beta = -0.30$: p = 0.04), but not Σ skinfolds ($\beta = -0.30$: p = 0.07), after adjustment for age and school clustering. No additional significant association were observed between SLT, SLT bouts of <30 minutes, SLT bouts of \geq 30 minutes, StT, LIPA (incl. StT) or MVPA and measures of adiposity after adjusting for age and school clustering(Table 3). Adjusting for MVPA in models, which included the other physical activity and sedentary behavior variables, did not improve the fit or change the conclusions from the models (results not shown).

Discussion:

This study examined the associations between the percentage of waking time spent in SLT, breaks in SLT, percentage of waking time spent in SLT bouts <30 and ≥30 minutes in duration, StT, LIPA (both incl. and excl. StT) and body composition measures in a sample of adolescent females using an inclinometer-based activity monitor. The use of this monitor allows the novel examination of StT as a separate activity variable to SLT and LIPA which has not been possible using alternative monitors. Of all of the physical activity and sedentary behavior variables measured in this study, LIPA (excl. StT) was identified as having the strongest association with both BMI percentiles and Σ skinfold thickness. These associations were independent of age. The number of breaks in SLT was also significantly associated with BMI percentiles, after adjustment for age. The association between breaks in SLT and BMI percentile indicates that replacing SLT with other activity is beneficial for BMI. This association mirrors the stronger association between LIPA and BMI percentiles. Together, these associations suggest that breaking SLT and replacing it with LIPA is associated with a reduced BMI percentile. No additional significant associations were evident between physical activity and sedentary behavior variables (including MVPA) and measures of body composition in this sample after adjusting for age.

The findings of this study have public health significance. The dramatic increase in the prevalence of overweight and obesity in developed nations has prompted great interest in developing alternative approaches to increase daily energy expenditure in sedentary lifestyles, including increasing the amount of time spent in StT (14). However, a recent examination of the energy expended during StT compared to SLT has identified that minimal differences in energy

expenditure exist (<20kcal/day) (19). These findings may help to explain the results of this study. Although a large proportion of waking time was spent in a standing posture (23.0%), the amount of energy expended due to standing may not be sufficient to positively impact body composition in this adolescent female sample. Additionally, when StT was included with LIPA (excl. StT), the behavior appears to mask the effect of LIPA (excl. StT) on measures of body composition. This study suggests that interventions which target increasing LIPA (excl. StT) (i.e. ambulation at an intensity of <3 METs) through reducing SLT and StT may change adiposity in an adolescent female population. It is important to note that the postural change from sitting to standing may have additional health benefits in relation to the breaking of prolonged sedentary time, but in this sample, StT, LIPA (incl. StT) or SLT bout duration were not associated with body composition measures.

Placing these findings among existing research is extremely difficult, primarily due to the lack of accurate information on objectively determined LIPA in child and adolescent samples. The predominant reason for this dearth of information is due to difficulties in detecting and assessing this specific activity behavior (25). Of the limited evidence on the associations between LIPA and adiposity in adolescent populations, the results are contrasting in nature (5, 26). Ekelund and colleagues identified no association between accelerometer determined LIPA (MTI activity monitor) and body fatness in 1292 9–10 year old European children, without adjusting for additional activity variables (e.g. SLT or MVPA) (5). In contrast, Steele et al. identified a weak but significant linear relationship between accelerometer determined LIPA (Actigraph GT1M activity monitor) and BMI after adjustment for a range of covariates including SLT (26). Differences observed across studies might be due to the inclusion of different covariates in the regression models, yet it is more likely due to the use of different sedentary thresholds, which

result in significant differences in the amount of quantified LIPA (10). As identified by Ridgers and colleagues, significant differences in accelerometer determined SLT can be observed using sedentary thresholds which differ greatly (23). Additionally, such accelerometer-based activity monitors have been shown to consistently and significantly overestimate sitting and walking activities, primarily due to their inability to examine StT, and consequently may have misquantified LIPA through the misclassifying StT as SLT or as LIPA (4, 6). Through the use of an inclinometer-based activity monitor, which accurately and reliably distinguishes between SLT and StT, this study has provided valid and reliable estimates of SLT, StT and LIPA, and has examined the associations between these physical activity and sedentary behaviors variables and adiposity.

The findings of this study have identified that SLT is not associated with body composition, after adjustment for age. The associations between SLT and body composition in this paper are consistent with existing examinations of the relationship between accelerometer determined SLT (which have corrected for MVPA) and cardiovascular risk factors in child and adolescent samples (2), but the present findings are based on SLT determined from an inclinometer-based activity monitor. Previous objective examinations have employed accelerometer-based activity monitors (e.g. ActiGraph GT1M and GT3X) as measures of physical activity, and have estimated SLT using an activity count threshold (e.g. <100 counts-min⁻¹). This method estimates SLT based on the lack of ambulation (23). In contrast, the present study has employed an inclinometer-based activity monitor, the activPALTM, which directly measures SLT through the inclination of the thigh. Significant differences have been observed between ActiGraph and activPAL determined SLT, with the activPAL demonstrating increased accuracy at measuring sedentary behaviors (6, 10). Furthermore, the use of the activPAL has been encouraged in studies

that aim to examine specific sedentary patterns and behaviors (1), while it has been employed as the reference measure for the objective examination of sedentary behaviors when validating accelerometer-based activity monitors (6, 23).

The findings presented here suggest that activities at the lower end of the physical activity continuum have an influence on health outcomes in adolescent females, and that increasing LIPA at the expense of SLT and StT may be of great benefit in the maintenance of a healthy weight profile in this population. Additional cross-sectional research is required to examine whether these associations are evident across all populations, including children, male adolescents and adults of all ages, while longitudinal and interventional evidence is necessary to determine the effects of reduced SLT through increased LIPA on adiposity and additional cardiovascular risk factors in all populations.

Limitations to this analysis include the cross-sectional design that represents a relatively small sample of adolescent females in one geographical area over a two year period, and may not be representative for all populations. We did not measure additional covariates, such as stage of pubertal development, nutritional information, socio-economic status, urban/rural dwelling and smoking to include in the models. Strengths of this study should be noted. To our knowledge, this is the first study to present relationships between the full range of physical activity intensities and sedentary behaviors including SLT, StT, LIPA and MVPA using an inclinometer-based activity monitor with adiposity in any population.

In summary, these observations have identified associations between LIPA (excl. StT) and body composition measures in an adolescent female population. The results of this study suggest that increasing LIPA (excl. StT) (e.g. slow walking, household chores etc.) at the expense of SLT and

StT could be a worthwhile initiative for weight management in an adolescent female population. Future interventional research should focus on whether decreasing total SLT through breaks and increasing LIPA may prevent unhealthy increases in adiposity in adolescent females. Further research is also required to examine and interpret the associations between breaks in SLT and body composition in adolescent females.

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	Mean (SD)	Range
Age (yrs.)	15.7 (0.9)	13.1 – 18.7
BMI $(kg/m^2)^{1}$	21.7 (5.2)	15.4 - 41.3
Sum of Skinfolds (mm) ²	62.0 (37.1)	26.6 - 207.1
Sitting/Lying Time (%)	65.3 (7.1)	44.3 - 83.0
Number of breaks in Sitting/Lying Time	59.7 (13.0)	32.0 - 106.4
Sitting/Lying Bouts less than 30 minutes (%)	34.8 (6.5)	8.7 - 49.9
Sitting/Lying Bouts greater than 30 minutes (%)	30.5 (8.8)	6.0 - 57.6
Standing Time (%)	23.0 (5.3)	10.6 - 41.0
Light Intensity Physical Activity incl. Stand (%)	28.7 (6.1)	13.8 – 47.6
Light Intensity Physical Activity excl. Stand (%)	5.6 (1.5)	2.5 - 11.2
Moderate to Vigorous Physical Activity (%)	6.1 (2.4)	1.6 – 13.6

Table 1. Descriptive characteristics of the sample (n=195).

¹ median (IQR); n = 194; ² median (IQR); n = 193;
(%) = Variables presented as a percentage of the waking day.

	BMI Percentiles ¹		Σ skinfolds	
	r _s	p-value	r _s	p-value
Sitting/Lying Time (%)	0.15	0.04	0.18	0.01
Number of breaks in Sitting/Lying Time	-0.10	0.18	-0.11	0.14
Sitting/Lying Bouts less than 30 minutes (%)	0.01	0.90	0.04	0.58
Sitting/Lying Bouts greater than 30 minutes (%)	0.10	0.16	0.10	0.18
Standing Time (%)	-0.10	0.17	-0.13	0.08
Light Intensity Physical Activity incl. Standing (%)	-0.15	0.03	-0.17	0.02
Light Intensity Physical Activity excl. Standing (%)	-0.24	0.001	-0.25	0.001
Moderate to Vigorous Physical Activity (%)	-0.04	0.57	-0.10	0.15

Table 2. Correlations between physical activity and sedentary behaviour variables and measures of adiposity (n=195).

 $^{1}n = 194$; (%) = Variables presented as a percentage of the waking day.

	Outcome: BMI percentile ¹		
	β (95% CI)	р	
Sitting/Lying Time (%)	0.51 (-0.01, 1.04)	0.06	
Number of breaks in Sitting/Lying Time	-0.30 (-0.59, -0.01)	0.04	
Sitting/Lying Bouts less than 30 minutes (%)	0.06 (-0.51, 0.64)	0.83	
Sitting/Lying Bouts greater than 30 minutes (%)	0.31 (-0.12, 0.74)	0.16	
Standing Time (%)	-0.43 (-1.14, 0.28)	0.23	
Light Intensity Physical Activity (incl. Standing) (%)	-0.57 (-1.17, 0.04)	0.07	
Light Intensity Physical Activity (excl. Standing) (%)	-4.38 (-6.87, -1.90)	0.0006	
Moderate-to-Vigorous Physical Activity (%)	-0.77 (-2.35, 0.81)	0.34	
	Outcome: Σ skinfold		
Sitting/Lying Time (%)	0.43 (-0.18, 1.03)	0.17	
Number of breaks in Sitting/Lying Time	-0.30 (-0.63, 0.03)	0.07	
Sitting/Lying Bouts less than 30 minutes (%)	0.11 (-0.56, 0.77)	0.76	
Sitting/Lying Bouts greater than 30 minutes (%)	0.22 (-0.27, 0.71)	0.37	
Standing Time (%)	-0.20 (-1.02, 0.63)	0.64	
Light Intensity Physical Activity (incl. Standing) (%)	-0.38 (-1.08, 0.33)	0.29	
Light Intensity Physical Activity (excl. Standing) (%)	-4.05 (-6.94, -1.16)	0.006	
Moderate-to-Vigorous Physical Activity (%)	-1.31 (-3.13, 0.52)	0.16	

Table 3. Association between physical activity and sedentary behaviour variables and measures of adiposity (n=195).

¹n = 194: BMI = Body Mass Index; Σ skinfold = Sum of four skinfolds;

Separate linear mixed models for each PA and SB variable with school as a random effect for BMI percentile;

Separate linear mixed models for each PA and SB variable with school as a random effect and age as a fixed effect for Σ skinfolds.