Objectively measured time spent sedentary is associated with insulin resistance independent of overall and central body fat in 9 to 10 year old Portuguese children

Luis B. Sardinha[1] PhD, Lars Bo Andersen[2] PhD, Sigmund A. Anderssen[2] PhD, Ana L. Quitério[1] MSc, Rui Ornelas[3] MSc, Karsten Froberg[4] PhD, Chris J. Riddoch[5] PhD and Ulf Ekelund[6,7] PhD

[1] Faculty of Human Movement, Technical University of Lisbon, Portugal
[2] Sports Medicine, Norwegian School of Sports Sciences, Oslo, Norway.
[3] University of Madeira, Portugal
[4] Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark.
[5] Sport and Exercise Science, Bath University, Bath, UK
[6] Medical Research Council Epidemiology Unit, Cambridge, UK
[7] School of Health and Medical Sciences, Örebro University, Örebro, SWEDEN

Correspondence:
Ulf Ekelund
MRC Epidemiology Unit
Institute of Metabolic Science
Addenbrooke's Hospital
Box 285
CB2 0QQ
Cambridge, UK
Ulf.Ekelund@mrc-epid.cam.ac.uk

ABSTRACT

Objective: We examined the independent relationships between objectively measured physical activity and insulin resistance in Portuguese children.

Research Design and Methods: School-based, cross-sectional study in 147 randomly selected girls (9.8 ± 0.3 years; 27.8 ± 9.3 % body fat) and 161 boys (9.8 ± 0.3 years; 22.0 ± 9.2 % body fat). Physical activity was assessed by the Actigraph accelerometer for 4 days and summarised as time spent sedentary (accelerometer counts < 500 /min), in light intensity (accelerometer counts 500-2000 /min) and moderate and vigorous intensity activity (accelerometer counts >2001 /min). We measured total and central fat mass by dual X-ray absorptiometry. Insulin resistance was expressed as the Homeostasis Model Assessment score.

Results: Time (min/d) spent sedentary was significantly and positively associated with insulin resistance (B–coefficient=0.001, 95% CI; 0.0002; 0.002, p=0.013). Time spent in MVPA (B–coefficient=-0.002, 95% CI;-0.003;-0.001, p=0.0009) and overall physical activity (B–coefficient=-0.001, 95% CI;-0.008; 0.003; p<0.0001) were significantly and inversely associated with insulin resistance. All associations remained statistically significant, although were attenuated after further adjustments for gender, birth weight, sexual maturity and total or central fat mass (p<0.03).

Conclusions: Physical activity is associated with insulin resistance independent of total and central fat mass in children. Our results emphasise the importance of decreasing sedentary behaviour and increasing time spent in moderate and vigorous intensity activity in children, which may have beneficial effects on metabolic risk factors regardless of the degree of adiposity.

The prevalence of childhood overweight and obesity is increasing at an alarming rate world wide (1-4). Excessive body fat is associated with numerous adverse health consequences, including impaired glucose tolerance, hyperinsulimenia and type 2 diabetes. Central obesity and especially visceral fat are recognised to predispose children and adolescents to the development of insulin resistance and type 2 diabetes (5, 6).

Physical activity may counteract the adverse effect of obesity on insulin resistance in children. Previous studies examining the associations between physical activity and insulin resistance have been conducted in overweight children (7), or in children predisposed to type 2 diabetes due to a family history of the disease (8). Furthermore, most previous observational studies (9-12) have used relatively imprecise self-report methods to assess physical activity which preclude the ability to quantify dose-response associations and determine the influence of different sub-components (time spent sedentary, and at light and moderate and vigorous intensity activity) of physical activity on insulin resistance. It is also unclear whether physical activity is associated with insulin resistance independent of overall and central adiposity (8, 10, 11, 12), and some have suggested a gender difference for the association between activity with insulin resistance (9).

We have previously shown that objectively measured overall physical activity is associated with insulin sensitivity in Danish 9 to 10 year old children (13). However, that study did not assess which of the sub-components of physical activity were related to insulin sensitivity, with skinfold measurements used as an indicator of overall adiposity. Dual X-ray absorptiometry (DXA) can be used to quantify both overall and central fat mass (FM) (14-16) which makes this method preferable to skinfold measurements.

Therefore, the aim of the present study was to examine the independent relationships between objectively measured physical activity and insulin resistance in Portuguese children.

MATERIALS AND METHODS

This is a cross sectional, population-based study in 9-10-year-old Portuguese children which was part of the European Youth Heart Study (EYHS), selection criteria has been reported elsewhere (17). A total of 530 healthy children from the county of Madeira, Portugal, were sampled and invited to participate in the study along with their parents. Of these, 72 children refuse to wear the activity monitor, 96 children were excluded due to not meeting the inclusion criteria (see below) and 37 children were excluded due to faulty activity monitors. Complete anthropometric, DXA body composition measurements, clinical and physical activity measurements were available in 308 (147 girls and 161 boys) children. There were no significant differences (p>0.05) in body weight, height, BMI, sum of skinfolds, waist circumference, fasting triglycerides, insulin, glucose, HDL cholesterol and cardio-respiratory fitness between those with complete data compared with the rest of children.

After written informed consent was obtained from a parent or guardian, and all data were collected at the University of Madeira.

Anthropometric and body composition measurements. Height was measured, without shoes, to the nearest 0.5 cm using a portable Harpenden stadiometer. Weight was measured to the nearest 0.1 kg with a calibrated beam balance scale while the children were wearing light clothing. Body mass index (BMI) was calculated as a weight (kg)/ height squared (m²). Overweight and obesity was defined according to age specific cut-points (18). Waist circumference was measured twice with a metal anthropometric tape midway between the lower rib margin and the iliac crest at the end of a gentle expiration, the average of the two measures was used for analysis. DXA was used to assess total and

regional body composition (QDR-1500, Hologic, Waltman, USA, pencil beam mode, software version 5.67 enhanced whole body analysis). Following the protocol for DXA described by the manufacturer, a step phantom with six fields of acrylic and aluminium of varying thickness and known absorptive properties was scanned alongside each subject, to serve as an external standard for the analysis of different tissue composition. Fat free mass (FFM) was defined as the sum of the fat-free soft tissue and total body mineral content from the whole-body scans. The same technician positioned the subjects, performed the scans, and completed the scan analysis according to the operator's manual using the standard analysis protocol.

Sexual maturation was assessed by the investigators, using Tanner's 5-stage scale for breast development in girls and pubic hair in boys (19). Children were stratified as pre-pubertal (Tanner stage 1) or having started puberty (Tanner stage 2 and 3). Data on birth weight, parental socio-economic status, body weight and height were collected by self-report.

Blood samples. After a 12-hour overnight fast, venous blood samples were taken from the antecubital vein and collected into EDTA vacutainer tubes. Samples were aliquoted and separated within 30 minutes and stored at –80 °C until transport to WHO certified laboratories for analyses (Cambridge, UK). Samples were analysed for serum glucose and insulin. Glucose was analysed using the Hexokinase method, measured on an Olympus AU600 auto-analyser for all samples (Olympus Diagnostica GmbH, Hamburg, Germany). Plasma specific insulin was determined by two-site immunometric assays with either ^{125}I or alkaline phosphatase labels. Cross-reactivity was <0.2% with intact proinsulin at 400 pmol/l and <1% with 32-33 split proinsulin at 400pmol/l. Inter-assay CVs were 6.6% at 28.6 pmol/l (n=99), 4.8% at 153.1 pmol/l (n=102) and 6.0% at 436.7 pmol/l (n=99), respectively.

The homeostasis model assessment (HOMA IR) was calculated by dividing the product of fasting glucose (mmol/L) and fasting insulin (micro units/mL) by 22.5 (20). This method serves as a valuable surrogate for the assessment of insulin resistance in non-diabetic children (21). We defined insulin resistance as HOMA-IR > 3.16 (22).

Assessment of Physical activity. Physical activity was assessed using the Computer Science & Applications (CSA) accelerometer, also known as the MTI Actigraph (Manufacturing Technology Inc., Fort Walton Beach, FL, USA), over 2 weekdays and 2 weekend days, as previously described (23). Briefly, the children wore the accelerometer attached to an elastic waist-band on the right hip, volunteers were instructed to wear the monitors during the daytime, except while sleeping, bathing and during aquatic activities. Activity data were stored on a minute-by-minute basis and were downloaded to a computer before analysis. Physical activity components, including wearing time, were derived as previously described (24), using a special written software (MAHUffe, www.mrc-epid.cam.ac.uk). We first deleted missing data defined as sequences of 10 or more of consecutive zeros. We thereafter excluded all children who did not manage to record ≥600 min/d of activity for ≥3d were excluded from the analysis. The following variables were derived from the software; total volume of physical activity (counts/min/d), time (min/d) spent in sedentary activities (<500 counts/min), at light (500-1999 counts/min), moderate (≥2000 counts/min), and vigorous (≥3000 counts/min) intensity physical activity. Children spent < 3% of the daytime at a vigorous intensity level. Therefore, time spent in moderate and vigorous intensity activities were combined into one single variable (MVPA). Our threshold for MVPA (>2000 counts/min) corresponds to a walking speed of about 3 to 4 km/h (21).

Statistical methods. Data are presented as means ± SDs. All variables were checked for normality. Fasting insulin and HOMA IR were logarithmically transformed to normalize their distribution. Differences in body composition variables, physical activity patterns, and biochemical variables between genders were determined by analysis of variance. Pearson's correlation coefficients were used to examine the bivariate associations between insulin resistance (HOMA IR) and each of the physical activity components. Multiple linear regression analysis was used to test the independent associations between physical activity variables and insulin resistance. Model building was performed by first assessing the associations between gender, sexual maturity and birth weight with insulin resistance, thereafter introducing the sub-components of physical activity in separate models, as some of these variables were highly correlated with each other (r>0.70). All physical activity sub-components were adjusted for the amount of time the monitor was worn. Finally, we adjusted our models for total fat mass or central fat mass assessed by DXA. We included the interaction term gender by physical activity separately for each physical activity component model in order to explore whether gender or sexual maturity modified any of the associations between physical activity and insulin resistance,. Similarly, a 3-way interaction term (gender x sexual maturation x physical activity) was also introduced into the respective models. Finally, we introduced socio-economic status and parental BMI, and substituted FM by waist circumference as potential confounding variables but adjustment for these variables did not change the magnitude or direction of associations and were therefore excluded from our final models. All data were analysed in their continuous form although stratified into quartiles of time spent sedentary and at moderate and vigorous intensity activity for illustrative purposes. Data were analysed using the Statistical Package for Social Sciences (SPSS, version 13.0 for WINDOWS; SPSS Inc., Chicago), and the level of significance was set at $p < 0.05$.

RESULTS

Descriptive characteristics are presented in **Table 1**. Age, weight, height, and BMI did not differ significantly between boys and girls. Total and regional body fat, percentage fat mass, fasting insulin, and HOMA IR were significantly higher in girls than in boys. Significant gender differences were also observed for physical activity components. Boys spent significantly less time at light intensity activity and more time at moderate and vigorous intensity physical activity than girls. Time spent sedentary did not differ significantly between boys and girls. 18.5 % of children (20.4% girls and 16.8% boys) were classified as overweight and additional 8.8% children (8.2% girls and 9.3% boys) were obese. Fifteen children (3 normal-weight, 3 overweight and 9 obese) were insulin resistant.

Time spent sedentary was significantly correlated with time spent at light intensity activity (r=-0.15, p=0.008) and with time spent at moderate and vigorous intensity activity (r=-0.70, p<0.0001). Time spent at light intensity activity was not correlated with time spent at moderate and vigorous intensity activity (r=0.05, p=0.50). Time spent sedentary was significantly and positively associated with fasting insulin (r=0.21; *p*<0.0001) and HOMA IR (r=0.21; *p*<0.0001). Time spent at moderate and vigorous intensity PA was significantly and inversely correlated with fasting insulin and HOMA IR (r=-0.23 and -0.24, respectively; p<0.0001). Similarly, total PA (counts/min) was also significantly and inversely associated with fasting insulin and HOMA IR (r=-0.27 and -0.28, p<0.0001). All associations were attenuated but still statistically significant after further adjustment for gender and monitored time (partial r= <0.05). Time spent at light intensity was not associated with fasting insulin (r=0.10; p=0.08) or HOMA IR (r=0.10; p=0.08) and these non-significant

associations remained after further adjustment for gender and monitored time.

Table 2 displays the regression coefficients (95% CI) for the association between sub-components of PA and insulin resistance. Time spent sedentary was significantly and positively associated with HOMA IR (p=0.013) after adjustment for gender, sexual maturity and birth weight. Total PA (p<0.0001) and time spent at moderate intensity activity (p=0.0009) were also significantly but inversely associated with HOMA IR after adjustment for the same confounders as above. No association was observed between time spent at light intensity activity and insulin resistance and no significant two or three-way interactions were observed for any of our models.

We thereafter adjusted our models for total FM or central FM (**Table 2**). These adjustments attenuated the associations between PA variables and insulin resistance. However, time spent sedentary was positively associated with HOMA IR after further adjustments for total or central FM (*p*=0.027), and time spent in moderate and vigorous intensity PA (p=0.011) and overall PA (p=0.007) were significantly and inversely associated with HOMA IR after adjustment for total or central FM. Physical activity variables explained 8% to 9% of the variance in HOMA IR with a full model including gender, sexual maturity, birth weight, and total FM, explaining between 32% and 34% of the variance in HOMA IR (data not shown). We thereafter substituted waist circumference for central fat mass and reanalysed our models but this did not materially change the results.

Figure 1 shows the adjusted geometric means of HOMA IR stratified by quartiles of time spent sedentary (Fig 1a; p for trend = 0.043), and time spent at moderate and vigorous intensity physical activity (Fig 1b; p for trend = 0.11). Post hoc analyses revealed significant differences (P< 0.05) between the first and fourth quartile for time spent sedentary and at moderate and vigorous intensity.

DISCUSSION

This is the first study suggesting that objectively measured time spent sedentary is associated with insulin resistance in 9 to 10 year old healthy, prepubertal children, independent of gender, birth weight, sexual maturity and total or central fat mass. Further, these associations were consistent for both continuous and categorical analyses and suggest that reducing sedentary behaviour and increasing the amount of time spent at moderate and vigorous intensity physical activity may have beneficial effects on insulin resistance in healthy children.

When interpreting the results from this study the following limitations need consideration. First, our study was cross-sectional, thus limiting inferences of causality and its direction. Secondly, although we controlled for the confounding effect of gender, sexual maturity, birth weight and total or central fat mass, it is possible that other unmeasured confounders such as genetic variation and dietary factors could explain our findings. Thirdly, our results may only be generalizable to Portuguese 9-10 year old children. However, given the increasing evidence of an independent association between objectively measured physical activity and metabolic risk factors in children and adolescents (13, 25, 26), it is likely that our results are generalizable to a large proportion of healthy children living in affluent societies. Even though we assessed physical activity using an objective and valid method (27), some limitations remain. The monitor must be removed during bathing and other water activities and it does not accurately pick up upper body movement and other activities that involve minimal vertical acceleration of the body, such as cycling. Furthermore, limitations are inherent in all intensity thresholds used to summarise accelerometry data (28), however, our threshold for moderate and vigorous intensity activity is consistent with our previous studies (23, 26). The threshold used for time spent sedentary is a compromise between the lowest (<100 cpm; 29) and highest (<1100

cpm; 30) published sedentary cut points. However, reanalysing our data using the lower cut point (<100 cpm) did not change any of our results. We used a 1 minute epoch when assessing physical activity by accelerometry, this may be a potential limitation as this epoch length may underestimate the amount of time spent in vigorous intensity activity in children (31). However, it is highly unlikely that this would change the direction of the observed associations but it may attenuate them. Regardless, the use of an objective method for assessing physical activity and its sub-components in this study must be considered as a major strength. Other strengths include our precise measure of total and central fat mass by DXA and the collection of fasting blood samples in a randomly selected population of young children. Our results also highlight the limitations of using BMI as a measure of body composition when comparing differences between genders. Indeed, FM and percentage body fat measured by DXA were significantly higher in girls than in boys whereas there was no difference in BMI. Furthermore, we did not observe any difference in height between genders, which may have contributed to the non-significant difference in BMI.

Nine children (5 girls and 4 boys) did not accumulate 60 minutes or more of moderate intensity physical activity according to current recommendations of health enhancing physical activity in youth (32, 33). However, we have recently suggested that physical activity levels may need to be higher than the current guidelines of 1 hour of moderate intensity physical activity per day (26). Our study was not powered to examine non-linearity; thus studies that seek to determine whether there is a threshold for the association between physical activity and insulin resistance in children are required. Regardless, these results support our previous findings (13), underscoring the importance of reducing sedentary time in relation to insulin resistance in children.

Evidence from exercise training studies in obese children suggests that moderate and vigorous intensity exercise is associated with a favourable metabolic profile (7). Some (9-13) but not all (8) previous observational studies have reported an independent association between physical activity and insulin sensitivity in children. For example, subjectively measured physical activity was significantly associated with fasting insulin and insulin sensitivity independent of age, gender, race, maturation, body mass index, percent body fat, waist circumference and lipid levels, in 10 to 16 year old children (10). Further, Ku and colleagues (34) observed an association between insulin sensitivity and vigorous intensity, but not with moderate intensity physical activity, independent of body fat and fat distribution. However, these studies assessed physical activity by self-report, which may limit the ability to accurately examine the influence of different sub-dimensions of physical activity, as these are likely to be reported with different degrees of error. Furthermore, the validity of self-reported physical activity in children is usually considered to be poor (35).

Previous studies from the EYHS have examined the association between objectively measured physical activity and metabolic risk factors in children (13, 25, 26). In these studies, an inverse association was observed between the total volume of physical activity and metabolic risk factors, independent of skinfold-assessed body fat. The results from the present study are novel, extending our previous observations. First, we examined the association between sub-components of physical activity and insulin resistance, with the results suggesting that time spent sedentary and at moderate and vigorous intensity activity are associated with insulin resistance in a dose-response manner. Secondly, our results suggest that these associations are independent of precisely measured total or central fat mass and other confounding factors including birth weight.

Identifying the detailed associations between sub-components of physical activity, insulin resistance and other metabolic disease risk factors is important to inform primary prevention and future interventions, aimed at increasing physical activity in children. Our observations are the first suggesting that objectively measured time spent sedentary is associated with insulin resistance in children and corroborate previous observations in overweight adults with a family history of type 2 diabetes (36). Similarly, Healy et al (37) also suggested that objectively measured time spent sedentary was positively associated with glucose intolerance in adult Australian healthy men and women using the same assessment method. Taken together, reducing time devoted to sedentary behaviours and simultaneously increasing the amount of time spent at moderate and vigorous intensity activity is likely to have significant metabolic health benefits not only in adults but also in healthy prepubertal children, regardless of their levels of adiposity. Interestingly, our results suggest that sedentary time should be replaced with moderate and vigorous intensity physical activity, but not with light intensity activity, as light intensity activity does not seem to confer any benefit in relation to insulin resistance. This may at least partly be explained by the large amount of time devoted to moderate and vigorous intensity activity in these children combined with a strong inverse correlation between moderate and vigorous intensity activity and time spent sedentary. From a public health perspective, this may mean that promoting activities such as active play, active commuting by walking or bicycling, sport and other outdoor leisure activities usually performed at a moderate intensity should be prioritised. This is in agreement with current recommendations for physical activity in children (32, 33).

In conclusion, time spent sedentary and at moderate and vigorous intensity physical activity is associated with insulin resistance independent of total or central fat mass in healthy Portuguese children. Reducing sedentary behaviour and increasing the totality of activity may have beneficial effects on metabolic risk factors, even in pre-pubertal children. The development and implementation of multidimensional strategies focused on reducing sedentary behaviors and increasing overall involvement in different types of moderate intensity activity are critically important in the primary prevention of metabolic disorders already at young age.

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TABLE 1. Physical characteristics of the children.

	All (n=308)	Boys (n=161)	Girls (n=147)
Age (yrs)	9.81 ± 0.3	9.81 ± 0.3	9.82 ± 0.3
Weight (kg)	34.1 ± 7.8	34.3 ± 7.7	33.9 ± 7.8
Height (m)	1.37 ± 0.1	1.37 ± 0.1	1.37 ± 0.1
BMI (kg/m²)	18.0 ± 3.1	18.1 ± 3.2	17.9 ± 3.0
Waist circumference (cm)	61.5 ± 7.3	63.4 ± 7.3	59.4 ± 6.6***
Birth weight (kg)	3.40 ± 0.52	3.46 ± 0.55	3.32 ± 0.48*
Total PA (counts/min)	654 ± 240	724 ± 268	577 ± 178***
Sedentary Activity (min.d⁻¹)	315 ± 90	307 ± 95	323 ± 84
Light Activity (min.d⁻¹)	287 ± 49	278 ± 47	296 ± 49***
Moderate and Vigorous Activity (min.d⁻¹)	177 ± 64	195 ± 67	157 ± 54*
Fat mass (kg)	9.10 ± 5.6	8.19 ± 5.6	10.1 ± 5.4*
Fat mass (%)	24.8 ± 9.4	21.9 ± 9.0	28.0 ± 8.8***
Trunk fat mass (kg)	2.82 ± 2.5	2.39 ± 2.5	3.29 ± 2.5*
Central fat mass (kg)	0.67 ± 0.6	0.59 ± 0.5	0.77 ± 0.5*
Glucose (mmol/L)	5.2 ± 0.3	5.2 ± 0.3	5.2 ± 0.4
Insulin (micro units/mL)	6.1 ± 3.5	5.3 ± 2.8	7.0 ± 3.9***
HOMA IR[a]	1.21 (1.10; 1.32)	1.09 (0.99; 1.19)	1.38 (1.26; 1.50)***

Data are means ± SD. *$p<0.05$; *** $p<0.001$; for gender differences

[a]Geometric means and 95% CI

TABLE 2. Multiple linear regression coefficients (95% CI) for the association between objectively measured components of physical activity with insulin resistance (HOMA IR) in Portuguese children (n=308).

Outcome: HOMA IR	Model 1		Model 2		Model 3	
	B–coefficient (95% CI)	p-value	B–coefficient (95% CI)	p-value	B–coefficient (95% CI)	p-value
Sex (girls)	0.370 (0.22; 0.52)	<0.0001	0.18 (0.04; 0.31)	0.01	0.20 (0.07; 0.33)	0.001
Sexual maturity (Tanner 1)	-0.410 (-0.59; -0.23)	<0.0001	-0.17 (-0.33; -0.004)	0.045	-0.19 (-0.35; -0.02)	0.025
Birth weight (kg)	-0.060 (-0.18; 0.06)	0.33	-0.14 (-0.24; -0.03)	0.013	-0.10 (-0.21; 0.006)	0.065
Fat mass (kg)			0.05 (0.04; 0.06)	<0.0001		
Central fat mass (kg)					0.48 (0.38; 0.59)	<0.0001
Total PA (cpm)	-0.001 (-0.008; -0.003)	<0.0001	-0.0003 (-0.0006; -0.00001)	0.007	-0.0003 (-0.0006; -0.0009)	0.007
Sedentary (min/d)	0.001 (0.0002; 0.002)	0.003	0.001 (0.000001; 0.002)	0.012	0.0008 (0.00009; 0.002)	0.009
Light (min/d)	0.0003 (-0.001; 0.002)	0.75	-0.0002 (0.002; 0.001)	0.81	-0.0002 (-0.002; 0.001)	0.76
MVPA (min/d)	-0.002 (-0.003; -0.001)	0.002	-0.001 (-0.002; -0.0003)	0.017	-0.001 (-0.003; -0.001)	0.022

Model 1 adjusted for sex, sexual maturity and birth weight;
Model 2 additionally adjusted for overall fat mass;
Model 3 additionally adjusted for central fat mass
Time variables are additionally adjusted for measurement time

FIGURE LEGEND

Figure 1. Adjusted geometric means of HOMA IR stratified by quartiles of time spent sedentary (**Fig 1a**; p for trend = 0.043), and time spent at moderate and vigorous intensity physical activity (**Fig 1b**; p for trend = 0.11) in 9-10-year old Portuguese children (n=308). Post hoc analyses revealed significant differences (P< 0.05) between the first and fourth quartile for time spent sedentary and at moderate and vigorous intensity. Data are adjusted for gender, sexual maturity, birth weight and fat mass.

FIGURE 1