

## PAPER

# Objectively measured physical activity correlates with indices of insulin resistance in Danish children.

## *The European Youth Heart Study (EYHS)*

S Brage<sup>1,2\*</sup>, N Wedderkopp<sup>1</sup>, U Ekelund<sup>2,3</sup>, PW Franks<sup>2</sup>, NJ Wareham<sup>2</sup>, LB Andersen<sup>4,5</sup> and K Froberg<sup>1</sup>

<sup>1</sup>Institute of Sport Science and Clinical Biomechanics, University of Southern Denmark, Main Campus, Odense University, Odense, Denmark; <sup>2</sup>MRC Epidemiology Unit/University of Cambridge, Cambridge, UK; <sup>3</sup>Department of Physical Education and Health, Örebro University, Örebro, Sweden; <sup>4</sup>Institute of Sport Science, University of Copenhagen, Denmark; and <sup>5</sup>Norwegian University of Sport and Physical Education, Oslo, Norway

**OBJECTIVE:** To explore the association between measures of insulin resistance with objectively assessed physical activity.

**DESIGN:** School-based, cross-sectional study.

**SUBJECTS:** A randomly selected sample of 589 children (310 girls, 279 boys, mean (standard deviations, s.d.) age = 9.7 (0.44) y, weight = 33.6 (6.4) kg, height = 1.39 (0.06) m) from Denmark.

**METHODS:** Fasting blood samples were analysed for serum insulin and glucose. Physical activity was measured with the uniaxial Computer Science and Applications (CSA) model 7164 accelerometer, worn for at least 3 days ( $\geq 10$  h day<sup>-1</sup>). Adiposity was assessed by the sum of four skinfolds. Multiple linear regression were performed to model insulin and glucose from average CSA output, adjusted for age, gender, puberty, ethnicity, birth weight, parental smoking, socioeconomic group, and CSA unit. In addition, we adjusted for skinfold thickness.

**RESULTS:** Mean fasting serum glucose ranged from 4.1 to 6.5 mmol l<sup>-1</sup> with a mean (s.d.) of 5.1 (0.37) mmol l<sup>-1</sup>. Fasting insulin was negatively correlated with CSA output on levels of adjustment. Fasting glucose was not significantly associated with physical activity. However, in girls both indices of insulin resistance were significantly related to activity, whereas in boys none of the associations were significant.

**CONCLUSION:** Physical activity is inversely associated with fasting insulin in the nondiabetic range of fasting glucose. The relationship was stronger for insulin than for glucose, indicating compensatory action by the  $\beta$  cells. Our data emphasise the importance of physical activity in children for the maintenance of metabolic control.

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### Introduction

In recent years, an increasing prevalence of type 2 diabetes mellitus and its intermediate traits has been observed in children.<sup>1–3</sup> Previous studies have demonstrated inverse relationships between measures of insulin resistance and subjectively measured physical activity in the paediatric population.<sup>4–7</sup> However, questionnaire-based assessment of physical activity, which is the most common subjective

method, is imprecise, particularly in children. Consequently, it is normally recommended that in children of 11 y of age or younger, self-report methods are not used.<sup>8–11</sup> A positive correlation between fasting insulin and doubly labelled water measured physical activity has been reported in a small sample of premenarcheal girls, although this relationship disappeared when outliers were excluded.<sup>12</sup> No epidemiological study has so far related insulin resistance in children to an objective measure of physical activity, for example, accelerometry. Therefore, the dose–response relationship between physical activity and insulin resistance in children remains unclear, making it difficult to derive informed public health recommendations. Thus, the aim of this study was to investigate the association between indices

\*Correspondence: Dr S Brage, MRC Epidemiology Unit/University of Cambridge, Strangeways Research Laboratories, Wort's Causeway, Cambridge CB1 8RN, UK.

E-mail: sb400@medschl.cam.ac.uk

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of insulin resistance and an accelerometry-derived measure of physical activity. These relationships were examined at multiple levels of adjustment for potential confounders, including age, gender, puberty, adiposity, weight, height, ethnicity, birth weight, socioeconomic grouping, parental smoking, and accelerometer unit. Furthermore, because of increasing interest in the association between birth weight and insulin resistance,<sup>13–15</sup> we also investigated whether birth weight modifies the relationship between physical activity and insulin resistance.

## Materials and methods

### Design

The study was a school-based, cross-sectional study of prepubertal children, randomly selected by a two-stage sampling strategy. Data were collected in the academic year 1997–1998 in the county of Odense, Denmark, as part of the European Youth Heart Study (EYHS).

### Participants

*Target population:* Altogether 711 8- to 10-y-old children, who attended schools in the county of Odense, Denmark, were invited to participate in the study, along with their parents.

*Sampling:* Schools were stratified according to location (urban, suburban, rural) and socioeconomic profile of uptake area (high, middle, low). From each stratum, a proportional, two-stage cluster sample of children was selected. The primary units were the schools. The sampling frame for schools was a complete list of public schools in Odense, from which schools were sampled using probability proportional to school size. The secondary units were the children within the schools, and equal numbers of children were sampled from each school. The children were allocated code numbers and randomly selected using random number tables.

*Study sample:* In all, 28 out of 35 schools were sampled, and 25 agreed to participate. The only two urban low-income area schools in Odense were sampled, 20 out of 24 schools from middle-income areas, two out of four schools in high-income areas, and four out of five rural area schools were sampled. Of the three nonparticipating schools, one was rural, one was urban from middleclass area, and one was urban from a low-income area. All these three schools gave interference with the educational process as reason for not participating. A total of 711 individuals (58% of the total eligible population) were sampled and invited to participate in the study, of which 690 children (97%) responded to the invitation. A total of 589 (83% of the sampled and 48% of the total eligible population) participated in the study together with their parents. Among the participating children, there were 310 females and 279 males, which reflected the gender distribution of the Odense region.

### Ethics

The study was approved by the local scientific ethics committee (case no. 96/272) and performed in accordance

with the Helsinki declaration. All parents gave written informed consent for their child to participate and all children gave verbal consent. The measurement procedures were carefully explained during meetings held at each school as well as in the written invitation.

### Data collection

*Physical examination:* Height and weight were measured with a stadiometer and a calibrated scale, respectively. The level of adiposity was assessed by the skinfold technique, which has been shown to correlate highly with DEXA measured body fat percentage in similarly aged children.<sup>16</sup> Harpenden callipers were used to measure skinfold thicknesses at the positions of the m.triceps brachii, m.biceps brachii, subscapularly, and superior to the spina iliaca anterior superior. Measurements were performed on the left side of the body with the child standing. Two measurements were taken on each position. If there was a difference of more than 2 mm, a third measurement was taken, and the mean of the two closest measurements was then used. Measurements were made in rotation (one measurement on each site, then repeated). All measurements were performed by trained personnel and quality control was frequently performed. The same male researcher measured all boys, whereas the girls were measured by two female researchers. Intra- and inter-researcher reliability was  $r > 0.925$  and  $r > 0.835$ , respectively. Puberty status was assessed by examination according to Marshall and Tanner.<sup>17,18</sup>

*Blood samples:* All children and their parents received written information and the children were asked to be fasted when attending the school for blood sampling. The majority of children when asked reported that they had not eaten breakfast. Two children stated that they had eaten breakfast. Therefore, arrangements were made to draw blood from these children the following morning, and the requirement that they attended the school fasted was reiterated. The fasting samples were taken between 0800 and 2030 in the morning. They were immediately stored at  $-80^{\circ}\text{C}$  and analysed at a central certified laboratory for insulin and glucose. Glucose was analysed using the Hexokinase method, measured on an Olympus AU600 autoanalyser (Olympus Diagnostica GmbH, Hamburg, Germany). Insulin was analysed using enzyme immunoassay (microtitre plate format, Dako Diagnostics Ltd, Ely, England). The coefficients of variation between batches were as follows: glucose 1.2% (at 3.26 and 14.67  $\text{mmol l}^{-1}$ ) and insulin 6.9% (at 110.4  $\text{pmol l}^{-1}$ ) and 5.9% (at 356.2  $\text{pmol l}^{-1}$ ).

*Physical activity:* Habitual physical activity was assessed with the Computer Science and Applications (CSA) accelerometer, now also known as the MTI actigraph (Manufacturing Technology Inc., Fort Walton Beach, FL, USA). The CSA is a uniaxial piezoelectric accelerometer with a dynamic range of  $\pm 2.13\text{g}$  and a frequency-dependent bandwidth filter, which can be regarded as a mathematical weighting function.<sup>19,20</sup> The CSA samples acceleration at 10 Hz and

integrates this over the user-defined epoch. In this study, the CSA was mailed to the children, preprogrammed to start at 0800 the following morning. The children were instructed to wear it for 4 days at all times, except during water-based activities or when sleeping. The epoch was set at 1 min, comprising an integral of 600 measurements for each data point. The CSA was returned by the children and data downloaded on the day of the physical examination. In order to distinguish true zeros from the zeros recorded when the monitor had been taken off, the field data were cleaned in the following manner: All CSA files were screened for periods of zero activity. Zero activity periods of 10 min or longer were interpreted as 'CSA not worn' and these periods were thus removed from the summation of activity. Given these criteria, the data were included if the child had accumulated more than 10 h of activity data per day for at least 3 days. Data are expressed as total counts per registered time (counts min<sup>-1</sup>) to yield a measure of average physical activity intensity. Although the CSA exhibits good intrainstrument reliability in mechanical settings, interinstrument differences have been reported.<sup>20,21</sup> Analyses are therefore also adjusted for CSA unit as a categorical variable. There were 64 CSA units in use in this study.

**Other information:** The socioeconomic group (SEG) of each parent was assessed via questionnaire. Both the education and the income level of each of the parents were coded according to the National Statistic Registry and then recoded into five-level scores, ranging from 1 to 5. A higher number indicates a higher level of education and income. These two scores were then averaged into the SEG score, which therefore has nine levels (1, 1.5, 2, ..., 5). The parent questionnaire also contained questions on birth weight (kg), ethnicity (1=Caucasian, 0=Other), and parental smoking (coded as 1=one or both parents smoke, and 0=none of the parents smoke).

## Statistics

The ethical committee did not approve any collection of information on nonparticipants. Hence, dropout analyses are performed by comparing differences between subjects with complete data on the primary exposure and outcome and subjects with one or more missing values. All variables were checked for normality. *Insulin* and *average CSA counts* were normalised by square root, *body weight*, *glucose*, and *skinfold thickness* were normalised by the reciprocal square root, *age* was logarithmically transformed, and *birth weight* was raised to the power of two.

Following a bivariate correlation analysis, multiple linear regression analyses were used for assessing the relationship between physical activity and indices of insulin resistance. The relationships were adjusted for age, gender, puberty, ethnicity, socioeconomic status, parental smoking, and birth weight. Additional adjustments were made for body mass index (BMI) and the sum of four skinfolds, separately. The regression analyses were clustered on school (primary

sampling unit) to produce robust standard errors. To investigate the possibility of effect modification by birth weight, the analyses were also stratified by below and above the median birth weight. The software package STATA, *Version 8.0* (Stata Corp, TX, USA) was used for all statistical analyses. The level of significance was set at  $P=0.05$ .

## Results

### Representativeness

In comparison with the National Statistic Registry, the distributions of both educational level and income of the parents were representative of the country as a whole. Likewise, the BMI of both genders did not differ from the age-specific BMI, estimated from school census data.<sup>22</sup>

### Data completeness

Owing to a limited number of accelerometers available in this study, it was only possible to assess physical activity in 427 children. Of this group, 384 (179 boys, 205 girls) had valid physical activity data, as determined by the criteria described in the Materials and methods section. Of the outstanding 43 observations, data were unavailable due to download error ( $n=9$ ) or were excluded due to instrument breakage ( $n=23$ ), too little registered time ( $n=10$ ), or distinct unphysiological pattern (ie >9 standard deviations higher than the median physical activity,  $n=1$ ). Table 1 displays descriptives of all measured variables for the sample as a whole and for the subsample with complete data in all variables. Biochemistry data were available in 525 children (247 boys, 278 girls). The mean fasting glucose was 5.1 mmol l<sup>-1</sup>, ranging from 4.1 to 6.1 mmol l<sup>-1</sup>. Complete data for age, gender, puberty, physical activity, biochemistry, skinfold thickness, ethnicity, SEG, parental smoking, and birth weight were available in 313 children. There were proportionally fewer non-Caucasians in the subsample with complete data ( $P=0.002$ ).

**Table 1** Baseline characteristics of Danish 9- to 10-y-old children in the EYHS

Variable	All	Complete data subsample
Age (y)	9.6 ± 0.4	9.7 ± 0.4
Gender distribution (m/f)	279/310	150/163
Ethnicity (Caucasian/other)	543/40	306/7*
Weight (kg)	33.6 ± 6.4	33.5 ± 6.2
Height (m)	1.39 ± 0.06	1.39 ± 0.06
Puberty (Tanner stage)	1.09 ± 0.23	1.09 ± 0.20
Sum of four skinfolds (mm)	36.8 ± 18	37.0 ± 19
Insulin (pmol l <sup>-1</sup> )	55.8 ± 33	54.9 ± 28
Glucose (mmol l <sup>-1</sup> )	5.12 ± 0.4	5.13 ± 0.4
Birth weight (kg)	3.35 ± 0.6	3.37 ± 0.6
Parental smoking (yes/no)	341/231	176/137
SEG, mother	2.6 ± 0.9	2.6 ± 0.9
SEG, father	3.1 ± 1.0	3.2 ± 1.0
Physical activity (counts min <sup>-1</sup> )	660 ± 233	659 ± 231

Data are ratios or means ± s.d. \* $P < 0.05$  for difference between the subsample and the whole sample (All).

### Unadjusted associations

In the unadjusted correlation analyses, fasting insulin was inversely associated with physical activity ( $r = -0.23$ ,  $P < 0.001$ ), SEG of the father ( $r = -0.18$ ,  $P < 0.001$ ) and the mother ( $r = -0.17$ ,  $P < 0.001$ ), and age ( $r = -0.10$ ,  $P = 0.017$ ). Insulin was positively correlated with body weight ( $r = 0.38$ ,  $P < 0.001$ ), glucose ( $r = 0.35$ ,  $P < 0.001$ ), skinfold thickness ( $r = 0.24$ ,  $P < 0.001$ ), puberty ( $r = 0.23$ ,  $P < 0.001$ ), and height ( $r = 0.12$ ,  $P = 0.005$ ). Fasting glucose was inversely correlated with physical activity ( $r = -0.12$ ,  $P = 0.028$ ), SEG of the father ( $r = -0.13$ ,  $P = 0.003$ ) and the mother ( $r = -0.10$ ,  $P = 0.022$ ), and age ( $r = -0.11$ ,  $P = 0.010$ ). On average, boys had  $0.90 \text{ pmol l}^{-1}$  lower insulin levels ( $P = 0.010$ ),  $0.11 \text{ mmol l}^{-1}$  higher glucose levels ( $P < 0.001$ ), and had accumulated  $127 \text{ CSA counts min}^{-1}$  more than girls ( $P < 0.001$ ). Fasting insulin was  $19.3 \text{ pmol l}^{-1}$  higher in the non-Caucasians ( $P = 0.022$ ).

### Association between physical activity and indices of insulin resistance

The PA regression coefficients with 95% confidence intervals for fasting insulin and glucose are displayed in Table 2. There was an inverse relationship between physical activity and insulin after adjustment for potential confounders. This remained after adjustment for both BMI (standardised PA  $\beta = -0.179$ ,  $P = 0.039$ ) and when skinfold thickness was used as indicator of adiposity ( $P = 0.049$ ). Puberty status was the only other significant predictor of fasting insulin ( $P \leq 0.048$ ). There were no significant associations between fasting glucose and physical activity. The only significant predictor of fasting glucose was ethnicity (lower in Caucasian children,  $P \leq 0.009$ ). The associations were similar when the analyses were restricted to the Caucasian children and in the strata below and above the median birth weight (3.40 kg). However, when we stratified for gender, the relationships in the girls ( $n = 163$ ) between activity and insulin resistance were significant for both insulin and glucose, and also following adjustment for adiposity. In boys ( $n = 150$ ), the relationships were no longer significant.

**Table 2** Prediction of insulin resistance from physical activity

Model	PA coefficient	95% confidence interval	P-value	$\Delta R^2$ by PA
<i>Fasting insulin:</i>				
Model 1a	-0.085 (-0.188)	-0.160; -0.009	0.030	0.020
Model 1b	-0.079 (-0.179)	-0.158; -0.000	0.049	0.019
<i>Fasting glucose</i>				
Model 2a	0.0004 (0.117)	-0.0002; 0.001	0.207	0.008
Model 2b	0.0004 (0.109)	-0.0003; 0.001	0.241	0.006

Insulin and average CSA counts were both normalised by square root. Glucose was normalised by the inverse of the square root. Models with suffix 'a' are adjusted for age, gender, puberty, ethnicity, birth weight, parental smoking, socioeconomic status, and CSA unit. Models with suffix 'b' are additionally adjusted for skinfold thickness.

### Discussion

Physical activity sampled over 3–4 days represents only a fraction of the time that may be relevant for influencing insulin resistance. Nonetheless, the variance in fasting insulin was significantly associated with the variance in accelerometry measured physical activity after adjustment for potential confounders. This association was independent of BMI and skinfold thickness. Gender-specific analyses revealed that this was strongest in the girls, whereas none of the associations we explored were significant in the boys. However, this may be due to a combination of effect modification and insufficient statistical power. In contrast to fasting insulin, the association between physical activity and glucose was insignificant, when all confounders were controlled for. However, in girls this relationship was significant and persisted even after adjustment for adiposity.

The precision of objective assessment of physical activity energy expenditure in children is superior to subjective methods,<sup>8–11,23</sup> but there are nonetheless some limitations, which should be highlighted. For example, cycling and swimming is not captured. CSA output is related to acceleration but not in a linear manner, as it also depends on movement frequency.<sup>20</sup> Step frequency therefore influences validity of the CSA as a measure of intensity in running and to a lesser extent in walking.<sup>24–26</sup> Even so, CSA output is highly correlated with intensity within the walking–jogging range, although accelerometry generally underestimates higher running intensities due to biomechanical limitations.<sup>25,26</sup> Furthermore, since larger individuals have lower step frequencies,<sup>27,28</sup> this may cause differential bias because body size also relates to puberty status and adiposity, which are both linked to insulin resistance.<sup>29–31</sup> Interestingly, when adjusting for BMI, the relationship between activity and insulin remained virtually the same, whereas when skinfold was used as the measure of adiposity, this association was attenuated. This may well be explained by a BMI correction for measurement error in the activity variable, as opposed to an adjustment for adiposity *per se*. Adiposity is likely to be on the causal pathway between activity and insulin resistance and adjustment will therefore remove some of the true association. Although chance, bias, and confounding could not explain this association, residual confounding from unmeasured factors may be important. Additionally, genetic factors may well modify the relationship between physical activity and insulin resistance in children.

The coefficient for insulin was  $-0.085$  on the transformed scale (square root). This equates to  $-1.0 \text{ pmol l}^{-1}$ ,  $-1.2 \text{ pmol l}^{-1}$ , and  $-1.4 \text{ pmol l}^{-1}$ , on the 25th, 50th, and 75th percentiles of fasting insulin, respectively. One unit increment on the transformed physical activity scale equates into increments of  $46 \text{ counts min}^{-1}$ ,  $51 \text{ counts min}^{-1}$ , and  $58 \text{ counts min}^{-1}$  for the 25th, 50th, and 75th percentiles, respectively. Using doubly labelled water data,<sup>23</sup> an increase of one unit on the transformed square root of counts  $\text{min}^{-1}$  scale results in an increase of  $6.5 \text{ kJ kg}^{-1}$  of daily physical activity energy expenditure (age and gender adjusted) or

211 kJ day<sup>-1</sup> for a child weighing 32.4 kg (median body weight of the sample). The dose–response estimate from this calculation agrees reasonably well with the estimate obtained in a recent cross-sectional study in 10- to 16-y-old individuals, in which a 26.3 pmol<sup>-1</sup> decrease in fasting insulin for a questionnaire-estimated increase in physical activity energy expenditure of 6231 kJ was reported.<sup>7</sup> A 211 kJ increase in daily energy expenditure from activity in this population would thus correspond to a 0.9 pmol<sup>-1</sup> decrease in fasting insulin.<sup>7</sup> The small difference between the two studies may be explained by underestimation of physical activity by the CSA, overestimation by the questionnaire, and/or differences between other characteristics of the two cohorts, such as pubertal status and body composition.

Other studies in children have also reported inverse relationships between physical activity and insulin resistance indices.<sup>5,32</sup> Only a single study has reported a *positive* relationship between doubly labelled water measured energy expenditure and insulin.<sup>12</sup> However, the relationship in this study disappeared when two outliers were excluded from the analysis of this relatively small sample. Moreover, the estimates of energy expenditure were not standardised for body size and the analyses were not adjusted for puberty or adiposity. Thus, the positive association may be attributable to confounding.

Several trials have demonstrated improvements in insulin sensitivity following exercise intervention programmes<sup>33–41</sup> and, moreover, that cessation of activity reverses the training-induced changes.<sup>37</sup> However, in one trial involving 10 weeks of exercise training in 24 obese black girls, no significant change in fasting insulin was observed but this may be explained by compensatory behaviour, since only self-reported vigorous physical activity and not overall volume of activity was increased.<sup>42</sup>

The cross-sectional nature of our study limits inference about direction of causality. Nonetheless, the observation that fasting insulin level is more consistently associated with physical activity, whereas fasting glucose level is less consistently associated with physical activity, may be explained by the way in which normal levels of blood glucose are maintained when insulin resistance develops. Although end-stage insulin resistance is characterised by  $\beta$ -cell exhaustion, in the very early stages, such as in childhood insulin resistance, the pancreas compensates for tissue resistance by excreting greater quantities of insulin.<sup>43</sup> The association between activity and insulin resistance is most likely explained by enhanced insulin action and noninsulin-stimulated glucose uptake through physical activity, whereby the insulin pathway is off-loaded. Indeed, improved insulin action and glycaemic control through physical activity are usually the product of synergism between numerous mechanisms involving the muscle, adipose tissue, liver, and endothelium. These mechanisms include upregulation of key proteins in the insulin cascade, for example, insulin receptor substrates, phospho-inositol-3-kinase, and glucose transporting proteins.<sup>44</sup> Other mechanisms are

upregulation of the rate-limiting enzymes hexokinase, citrate synthase, and glycogen synthase, which would facilitate maintenance of the concentration gradient during the time when glucose transporting proteins are incorporated in the cell membrane. Another pathway may be the lowering of lipid levels in blood and muscle, which could otherwise impair the insulin cascade.<sup>45,46</sup> Existence of a causal relationship between physical activity and insulin resistance is further supported by the observation that immobilisation results in worsening of the glucose tolerance.<sup>47</sup>

In conclusion, physical activity is inversely associated with insulin resistance in glucose-tolerant Danish children and most evidently in girls. Our observations support the view that in children physical activity is an important behaviour for metabolic function. Therefore, the development and implementation of strategies to prevent sedentary behaviour in children is likely to be of paramount importance if metabolic disease is to be averted. Such lifestyle intervention strategies have successfully produced beneficial secular trends in insulin parameters in adolescents in a high school setting.<sup>48</sup>

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