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Published in:
International Journal of Obesity

DOI:
10.1038/s41366-018-0063-8

Publication date:
2018

Document version:
Accepted manuscript

Citation for pulished version (APA):

Chinapaw, M., Klakk, H., Møller, N. C., Andersen, L. B., Altenburg, T., & Wedderkopp, N. (2018). Total volume versus bouts: prospective relationship of physical activity and sedentary time with cardiometabolic risk in children. *International Journal of Obesity*, 42(10), 1733-1742. <https://doi.org/10.1038/s41366-018-0063-8>

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Total volume versus bouts: Prospective relationship of physical activity and sedentary time with cardiometabolic risk in children

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Running title: Activity patterns and child health effects

Conflict of interest:

The contributions of Altenburg and Chinapaw were funded by the Netherlands Organization for Health Research and Development (ZonMw; projectnr 91211057). The authors have no other relevant conflicts of interest to disclose.

Abstract

Background/Objectives: Examine the prospective relationship of total volume versus bouts of sedentary behaviour (SB) and moderate-to-vigorous physical activity (MVPA) with cardiometabolic risk in children. Additionally, the moderating effects of weight status and MVPA were explored.

Subjects/Methods: Longitudinal study including 460 primary school children (mean age 9.4 yrs). Total volume and bouts (i.e. ≥ 10 min consecutive minutes) of MVPA and SB were assessed by accelerometry in Nov 2009/Jan 2010 (T1) and Aug/Oct 2010 (T2). Triglycerides, total cholesterol/HDL cholesterol ratio (TC:HDLC ratio), homeostatic model assessment of insulin resistance, systolic blood pressure, and waist circumference were assessed at T2 (Sept/Oct 2010) and combined in a composite cardiometabolic risk score. Associations of total time and uninterrupted MVPA and SB were examined using multilevel mixed linear models, with or without mutual adjustments between MVPA and SB. The moderating effects of weight status and MVPA (for SB only) were examined by adding interaction terms.

Results: Children engaged daily in about 60 minutes of total MVPA and 0 to 15 minutes per week in MVPA bouts. Mean total sedentary time was around seven hours/day with over 3 hours/day accumulated in bouts. Higher mean levels of MVPA were significantly associated with a lower waist circumference, triglycerides, insulin resistance, TC:HDLC ratio and composite cardiometabolic risk, with non-significant associations for uninterrupted MVPA. Associations with sedentary time were much smaller and inconsistent: higher total sedentary time was associated with higher insulin resistance; after adjusting for MVPA, higher mean total and bouts of sedentary time were associated with lower waist circumference, and sedentary bouts with lower composite cardiometabolic risk.

Conclusions: Children accumulated MVPA intermittently and rarely in bouts and about half their total sedentary time in bouts. Total MVPA is important for lowering cardiometabolic risk in children, while both total and uninterrupted sedentary time seem of less importance.

Introduction

Physically active children and adolescents generally have better cardiometabolic health than their inactive peers¹. The evidence in youth for an association of sedentary behaviour with cardiometabolic health indicators (e.g. blood pressure, glucose and lipid levels) is limited and inconsistent²⁻⁵. A recent review of prospective studies concluded no evidence for a relationship of TV viewing time with triglycerides and glucose; and insufficient evidence for a relationship with the remaining cardiometabolic biomarkers⁴. This evidence is urgently required for optimal health promotion since today's children spend a large part of their time sedentary all around the world⁶⁻⁸. Moreover, physical activity levels decline and sedentary behaviour increases throughout childhood into adulthood^{9, 10}.

Adult studies suggest that especially uninterrupted sitting may be detrimental for cardiometabolic health¹¹⁻¹³. The few experimental studies on the acute effects of uninterrupted sedentary time in youth show mixed results. Saunders et al¹⁴ found no effects of a single bout of 8 hours of uninterrupted sitting in comparison to interrupted sitting on postprandial levels of insulin, glucose or lipids in healthy 10-14 year olds. In contrast, Belcher et al¹⁵ found that interrupting 3 hours of sitting with brief moderate-intensity walking improved insulin, c-peptide and glucose levels in normal-weight 7–11 year olds. Fletcher et al¹⁶ explored the impact of uninterrupted sitting and breaking up sitting (2-min resistance-type activity breaks every 18-min) on adolescents' postprandial glucose while consuming a diet varying in energy. Their findings were inconstant: the activity breaks significantly attenuated the postprandial iAUC responses when examining the first and second meal responses separately, but not for the entire trial period nor for when total AUC was examined. Few cross-sectional studies have examined the association between sedentary bouts and cardiometabolic risk in children. Carson et al¹⁷ found no association between overall volume and sedentary bouts with cardio-metabolic risk factors in a large sample of US children and adolescents (6-19 year olds). In a Canadian sample of 11-year olds sedentary bouts of 5–9 ($\beta = 0.22$, 95%CI: 0.01; 0.43) and 10–19 minutes ($\beta = 0.30$, 95%CI: -0.05; 0.55) were associated with BMI z-score, but only in the group with the lowest levels of MVPA¹⁸. Altenburg et al¹⁹ found few weak but significant associations of sedentary bouts of ≥ 5 min with cardiometabolic health indicators in European 10-13 year olds ($\beta = 0.002$, 95%CI 0.000; 0.003 for waist circumference and $\beta = 0.0002$, 95%CI: 0.0000; 0.0003 for C-peptide). To the best of our knowledge, no longitudinal observational studies have examined the association of moderate to vigorous physical activity (MVPA) or sedentary behaviour accumulated in bouts with cardiometabolic risk in youth^{3, 5}. As longitudinal studies provide a stronger case for causality they are urgently needed to unravel the health effects of various MVPA and SB patterns.

In youth, any lifestyle-related exposure may not have existed long enough to progress to subclinical or clinical expressions of disease, but children may be in the developmental stages of metabolic syndrome. Therefore, we examined associations with continuous cardiometabolic health indicators and a clustered cardiometabolic risk score^{20,21}. Composite cardiometabolic risk has been shown to track into adulthood²².

The association between sedentary behaviour and MVPA may be different in certain subgroups. As childhood obesity contributes to an increased prevalence of cardiometabolic risk factors, such as hypertension, dyslipidemia, and impaired glucose metabolism²³⁻²⁵, associations between physical activity and sedentary time with cardiometabolic risk may be stronger in overweight children.

Therefore, the current study examined the prospective relationship of total and uninterrupted MVPA and sedentary time with cardiometabolic risk in Danish primary school children aged 6-12 years followed for 1 year. Additionally, the moderating effect of weight status was explored and for sedentary time also the moderating effect of MVPA.

Methods

Design and participants

This study used data from the CHAMPS-study DK, which has previously been described in detail elsewhere²⁶. In 2007, all 19 public primary schools in the municipality of Svendborg in Denmark were invited to participate in a natural experiment of additional physical education lessons, of which six agreed to participate. Four control schools following the regular curriculum were recruited matched according to school size and socio-economic status. All children and parents from kindergarten to 4th grade (age range 5.5-12 years) were asked to participate in the study. In the intervention schools n=697 (90%) and in the control schools n=521 (71%) agreed to participate. Most children who left the study did so because they moved from the municipality. Only two children left the study because their parents disapproved of the study. For the current study we used accelerometer data from T1 (Nov 2009/Jan 2010) and T2 (Aug/Oct 2010), and cardiometabolic indicators at follow up (Sept/Oct 2010, T2: blood samples, blood pressure and waist circumference). A total of 857 children had accelerometer data at two time points; n=690 to 845 children had cardiometabolic indicators data at follow-up. N=454 children had complete data on at least one cardiometabolic indicator, exposure at both time points, and covariates and were included in the analyses. The study was carried out in accordance with the Declaration of Helsinki and was approved by the local scientific ethics committee (ID S20080047) and registered in the Danish Data Protection Agency (J.nr. 2008-41-2240).

Parents of the children gave written informed consent and the children gave verbal consent and could at any time withdraw from the project.

Measurements

A detailed manual was written for all measurements obtained and the researchers trained all the test-personnel (Master and PhD students) prior to each measurement round. Test-personnel's skills were tested at a non-participating school before data-collection. All measurements were performed at school.

Cardiometabolic health indicators

Waist circumference (WC) was measured with a Seca 201 Girth Measuring Tape to the nearest 0.5 cm at the level of the umbilicus with the child's abdomen relaxed at the end of a gentle expiration and used as a measure for abdominal fat deposit. Two measurements were performed except in the event of a discrepancy greater than 1 cm in which case a third measurement was performed. The average of the two nearest measurements was used in the analyses.

Blood pressure was recorded with a suitable cuff size on the left arm using an automated blood pressure machine (Welch Allyn®, Vital Signs Monitor, 300 Series with FlexiPort™ Blood Pressure Cuff 9). The child was resting in the sitting position for 5 minutes before monitoring. Five subsequent values were recorded with one minute intervals or until the last three values had become stable. Mean of the last three recordings was used in the analyses.

Pediatricians or pediatric laboratory assistants obtained fasting venous blood samples between 8:00 and 10:30 AM. Samples were kept on ice and handed in the laboratory within 4 h, pipetted and centrifuged, and kept at -80°C until analyzed. Total cholesterol (TC), triglyceride, high-density lipoprotein cholesterol (HDL-C), and glucose were analyzed by quantitative determination using enzymatic, colorimetric method on Roche/Hitachi cobas c system (Roche, Mannheim, Germany) in a certified laboratory at the University of Vienna. LDL-C was measured but we decided to include one indicator of cholesterol metabolism in the composite risk score (i.e. the TC:HDL ratio) as including all lipid variables would give lipids too much weight in the composite score. Previous studies showed that the TC:HDL ratio is a superior indicator of cardiovascular disease compared to single lipid markers²⁷. Total Cholesterol:HDL-C ratio (TC:HDL-C ratio) was calculated. Insulin was analyzed using solid phase enzyme-labeled chemiluminescent immunometric assay. Insulin resistance was assessed by a Homeostasis Assessment Model (HOMA-IR) score calculated from $\text{insulin } (\mu\text{U/ml}) \times \text{glucose } (\text{mmol/l}) / 22.5$ as described by Matthews et al²⁸.

Physical activity and sedentary behaviour

MVPA and sedentary time were assessed with the GT3X ActiGraph accelerometer (Pensacola, Florida, USA) using the vertical axis and standard filtering using a 2-second epoch. The research staff personally delivered the accelerometers to the children at the schools, placing it at the right hip using customized elastic belts. Written information and instructions were given to children and their parents. The children were instructed to wear the device from the time they woke up in the morning until bedtime for at least seven consecutive days, except when showering or swimming. Accelerometer data were analysed using a customized software program developed in R. For inclusion in the data analysis, each participant needed a minimum of six days with at least eight valid hours, including at least one weekend day. First, periods of more than 60 min of consecutive zero counts were defined as non-wear time and excluded from analysis. These data reduction decisions are recommended for analysis of sedentary behaviour in youth²⁹. We selected a cut-point of <100 counts per minute (cpm) to define sedentary time^{30, 31}. Total sedentary time (min/day) was calculated by summing all minutes < 100 cpm. Uninterrupted sedentary time was defined as all periods of ≥ 10 consecutive minutes < 100 cpm allowing no minutes above 100 cpm within a sedentary bout³². For SB no tolerance was allowed since the hypothesised mechanism is that a prolonged lack of muscle contractions suppresses glucose and fat metabolism³³. As any interruption in SB is accompanied by muscle contractions we did not allow tolerance in sedentary bouts, as this could be considered a sedentary break. Total MVPA was defined as each 15-second epoch above a cut-point of 574 counts/15 seconds³⁴. Uninterrupted MVPA was defined as all periods of ≥ 10 consecutive minutes ≥ 574 counts/15 seconds, allowing 10% of time below this threshold. For MVPA bouts tolerance was allowed, as heart rate will remain high when an MVPA bout is shortly interrupted so this is expected to negligibly influence potential cardiometabolic health effects. However, when examining for example the effects on bone strength, allowing less or no tolerance in MVPA bouts is reasonable as biomechanical strain is the underlying biological mechanism. Although physical activity guidelines for youth do not include that MVPA needs to be accrued in bouts of a certain duration, we used a minimum bout duration of 10 minutes as this has been included in adult guidelines³⁵.

Moderators

Body height was measured to the nearest 0.5 cm using a portable stadiometer (SECA 214, Seca Corporation, Hanover, MD), and body mass was measured to the nearest 0.1 kg using an electronic scale (Tanita Corporation, Tokyo, Japan). Assessments were performed barefooted and with children wearing shorts and t-shirt. Body mass index (BMI) was calculated as $\text{weight}/\text{height}^2$ (kg/m^2) and the cut-points for overweight proposed by the International Obesity Task Force were used³⁶.

Covariates

The Tanner pubertal stages self-assessment questionnaire³⁷ was used to determine pubertal status. Boys were presented with five pictures and text of Tanner staging for pubic hair development, whereas girls were presented with five pictures and text representing breast development and pubic hair³⁸. For our analyses we used pubic hair for boys and breast development for girls. Outcomes were reported on a 1 through 5 scale, with higher scores indicating later pubertal stages. Parental level of education was obtained by asking the parents their highest degree of completed education and collapsed into five levels: 1) primary and lower secondary education; 2) general upper secondary education; 3) vocational education and training; 4) bachelor degree; and 5) masters or PhD degree.

Statistical analyses

All analyses were conducted using SPSS Statistics 20. Descriptive characteristics were compared between normal weight and overweight (including obese) children using two-sample t-tests or Pearson's chi-squared tests. A relative composite cardiometabolic risk score was computed as the mean of the standardised scores from the following variables: WC, systolic blood pressure, TC:HDL ratio, log transformed HOMA, and triglycerides²². Each of these variables was first standardised (individual value – group mean)/standard deviations (SD)), stratified by gender. Using a continuously distributed composite risk score makes more sense in a sample of young and healthy children, since prevalence of deviating values will be low. Moreover, using a continuously distributed composite risk score is more sensitive and less susceptible to errors than dichotomous approaches and maximizes statistical power³⁹.

Multilevel linear mixed models with school and class as random effects were used to examine the association between the exposure variables (sedentary behaviour and MVPA by wear time) assessed at T1 and T2 and standardised scores of the cardiometabolic indicators at T2. First, residual change scores in exposure were analysed. The residual change score is the follow-up score adjusted for the baseline score and therefore represents change in exposure adjusted for baseline values. Second, mean exposure over T1 and T2 was analysed. Both models were adjusted for age, gender, parental education, puberty and schooltype (i.e. intervention or control) and subsequently also with mutual adjustment for MVPA and sedentary time. For example with MVPA as exposure the model was adjusted for sedentary time. The MVPA coefficient from this model can be interpreted as the effect of increasing MVPA on the expense of light intensity PA when all children would have the same amount of sedentary time. The mutually adjusted models can be interpreted as the association of the change and mean in relative exchange of time between light intensity PA and sedentary behaviour/ MVPA, respectively over the one-year follow-up period and cardiometabolic risk. The moderating effect of weight status at T1 was explored by assessing the statistical significance ($p < 0.10$) of the

interaction term between weight status (overweight versus normal weight) and residual change scores or mean exposures over T1 and T2, respectively. The moderating effect of MVPA status was explored by assessing the statistical significance ($p < 0.10$) of the interaction term between mean MVPA over T1 and T2 (highest versus the three lowest quartiles combined) and residual change scores or mean exposures over T1 and T2 of total and uninterrupted sedentary time by wear time, respectively.

Results

At baseline children were on average 10.3 years old, with 16% classified as being overweight or obese (Table 1). The mean number of valid accelerometer measurement days was 7.4 and valid wear time 12.8 hours/day at both time points. Normal weight children engaged daily in about 60 minutes of MVPA and overweight children in about 50 minutes MVPA. MVPA accumulated in bouts was rare with a median ranging from 0 minutes per week at baseline to 15 minutes per week at follow-up. Both total PA (counts per minute) and MVPA were significantly higher in normal weight than overweight children both at baseline and follow-up, while uninterrupted MVPA was significantly higher in normal weight children at follow-up only. Mean total sedentary time was around seven hours/day and mean uninterrupted sedentary time over 3 hours/day. Both mean total and uninterrupted sedentary time were similar in normal weight and overweight children. Overweight children scored significantly worse on all cardiometabolic health indicators (Table 2).

Table 3 presents the prospective relationship of both residual change and mean MVPA and sedentary time with the standardized individual cardiometabolic health indicators and the composite metabolic risk score adjusted for age, gender, parental education, puberty and schooltype. Higher mean levels of MVPA were significantly associated with a lower waist circumference, triglycerides, insulin resistance, TC:HDLC ratio and composite cardiometabolic risk, with non-significant associations for MVPA accumulated in bouts. Total and bouts of sedentary time were not significantly associated with any cardiometabolic indicator, except for change in total sedentary time being associated with insulin resistance.

Table 4 presents the prospective relationship of both residual change and mean MVPA and sedentary time with the standardized individual cardiometabolic health indicators and the composite metabolic risk score adjusted for age, gender, parental education, puberty and schooltype as well as mutual adjustment for MVPA and sedentary time. Residual change as well as mean total MVPA was significantly inversely associated with WC, systolic blood pressure and the cardiometabolic risk score, i.e. more active children had better cardiometabolic health. Mean MVPA was additionally associated with lower insulin resistance. For MVPA accumulated in bouts the effects sizes were much larger but not significant, except for the association between mean MVPA and systolic blood pressure.

Remarkably, total and uninterrupted sedentary time (both residual change and mean exposure) were also inversely associated with WC: children with higher sedentary time had a smaller waist circumference. Mean total sedentary time and sedentary time in bouts were additionally inversely associated with the cardiometabolic risk score. There were no consistent moderating effects by overweight or MVPA: For the moderation analyses by weight status one of 48 analyses showed a significant interaction of weight status by mean MVPA in the model with TC: HDLC-ratio (β for interaction: -15.9, 95%CI -30.5; -1.3, $p=0.02$). For the moderation analyses by MVPA only one of 24 analyses showed a significant interaction of MVPA by mean sedentary time in the analysis with LogHOMA (β for interaction: 0.26, 95%CI 0.05;0.48, $p=0.03$). Therefore, we decided against stratification.

Discussion

Children spending more time in MVPA over time had a smaller WC, triglycerides, insulin resistance, TC:HDLC ratio and composite cardiometabolic risk, with associations for MVPA accumulated in bouts being stronger but not significant, except for systolic blood pressure. No consistent associations between total and bouts of sedentary time and cardiometabolic risk indicators were observed. Remarkably, after adjustment for MVPA more total and uninterrupted sedentary time was associated with smaller waist circumference and a lower cardiometabolic risk score.

To our knowledge, no previous studies have assessed the prospective association between MVPA or sedentary time accumulated in bouts and cardiometabolic risk in children, and only a few assessed this for accelerometer-based total volume of physical activity⁴⁰⁻⁴² or sedentary time⁴². Ried-Larsen et al⁴⁰ found no significant association between 6-year change in MVPA and a metabolic risk z-score in adolescence in a sample of 254 Danish children participating in the European Youth Heart Study aged 8-10 years at baseline. However, the mean minutes of vigorous activity across childhood were associated with metabolic risk z-score in adolescence. Jago et al⁴¹ found in the same cohort that reductions in total physical activity (cpm) and MVPA between ages 9 and 15 were associated with higher fasting insulin levels and HOMA-IR levels at age 15. Hjorth et al⁴² examined the relationship between changes in MVPA and sedentary time with changes in a similar metabolic syndrome score over 200 days in 8-11 year old Danish children. Analogous to our findings they found a significant association between changes in MVPA with a similar composite metabolic risk score, but no association with sedentary time. In our sample MVPA accumulated in bouts of at least 10 minutes was rare confirming the spasmodic activity patterns of children this age. This may explain the lack of a significant association between uninterrupted MVPA and cardiometabolic risk although the regression coefficients were considerably higher than for total MVPA.

We chose to focus on bouts of sedentary time and not breaks for three reasons: i) The hypothesised mechanism of the potential detrimental effects of sedentary behaviour is a prolonged lack of muscle contractions³³. This is optimally reflected by examining bouts of uninterrupted SB; ii) Focusing on breaks would shift the focus to health effects of PA instead of sedentary behaviour; iii) Operationalising breaks raises many questions such as: should a break occur in between two sedentary bouts? If all counts above the sedentary cut-point are defined as breaks, you are actually analysing PA, both LPA and MVPA. If not all counts above the sedentary cut-point are included, should there be a maximum duration of a break? If yes, after how many minutes does a break become a physical activity bout? Should we use a minimum or maximum intensity for a break? When focusing on the frequency of breaks one hour of sports participation is equalled to standing up shortly. Moreover, the frequency of breaks equals the frequency of physical activity of at least light intensity and thus the focus would still be on physical activity. Because of all these questions there is no straightforward way or justification to analyse breaks in addition to sedentary bouts³².

The lack of an adverse and sometimes even beneficial association between sedentary time and the cardiometabolic indicators may be explained by the high levels of physical activity in our sample. The sample of similar aged Danish children in the study of Hjorth et al⁴² had lower mean counts per minute (487 versus 519 cpm in our sample) and time spent in MVPA (48 versus 59 min/day in our sample). Apparently, in physically active children sedentary time is of little importance. This is in line with a recent meta-analysis by Ekelund et al⁴³ showing that high levels of MVPA eliminate the increased risk of death associated with high sitting time among adults. However, this meta-analysis was based on self-reported sitting at one time point only. The few cross-sectional studies that have examined the associations between sedentary bouts and cardiometabolic risk indicators also found few significant associations^{17, 19}. Another explanation for the counterintuitive inverse association between sedentary time and waist circumference and cardiometabolic risk score after adjustment for MVPA may be that the children frequently interrupted their sedentary time as sedentary time accumulated in bouts of at least 10 minutes added up to just three hours per day. A third explanation maybe that even when sedentary children still engage in some body movement which may counteract the potential detrimental effects of sitting completely still (unpublished personal data). Future research should explore if the positive association between sedentary behaviour and waist circumference, after adjustment for MVPA, can be replicated, also in a less active sample. A final explanation could be measurement error as accelerometers are no gold standard for assessing sedentary behaviour.

The mutually adjusted models can be interpreted as the association of the change and mean in relative exchange of time between light intensity PA and sedentary behaviour/MVPA, respectively over the one-year follow-up period and cardiometabolic risk. The changes in effect sizes after mutual adjustment for MVPA and sedentary behaviour suggest that we should move towards the analysis of behavioural patterns rather than daily or weekly averages of a single behaviour, which is current practice. Our analyses of MVPA and sedentary behaviour in bouts is a first step in that direction. Future research needs to explore the optimal behavioural pattern for health benefit and answer questions such as: should MVPA be accumulated in bouts? Should sedentary behaviour be regularly interrupted and how often? Are health effects of 60 minutes a day similar to 210 minutes twice a week?

No previous studies examined the moderating effect of weight status or MVPA in children. The lack of a moderating effect of overweight may be due to the similarity in sedentary behaviour and small differences in MVPA between normal weight and overweight children. The lack of a moderating effect by MVPA may be explained by the above mentioned high levels of physical activity in our sample: even in the lowest quartile the mean MVPA was still 38 minutes/day. Both may have underestimated a potential association of MVPA and SB with cardiometabolic risk.

The main strengths of this study are the high response rate and compliance with accelerometer instructions increasing the generalisability of our findings. Other strengths are the objectively measured sedentary behaviour and physical activity and the inclusion of uninterrupted MVPA and sedentary time as well as availability of measures of cardiovascular risk factors in a large population of children. Moreover, this is one of the few studies on this topic with a longitudinal design. As children are generally metabolically healthy and cut-points in single metabolic risk factors are not established in children we used a composite score. A high level in the composite risk score is an undesirable condition, and has been shown to track into young adulthood⁴⁴. A limitation is the inability of accelerometers to capture cycling, swimming, and load-bearing activity and to separate sitting from standing as well as the lack of consensus on accelerometer cut-points and data reduction. Although we adjusted for a number of important confounders, we were not able to adjust for diet.

In conclusion, children accumulate their MVPA predominantly in short periods less than 10 minutes and about half of their total sedentary time in bouts longer than 10 minutes. All MVPA is important for lowering cardiometabolic risk in children, regardless of whether it is accumulated in bouts. Both total and uninterrupted sedentary time seem of less importance at this age. In children, promotion of

MVPA seems more important to reduce cardiometabolic risk than limiting sedentary behaviour. Future longitudinal research is recommended to further explore optimal behavioural patterns for cardiometabolic health.

Conflict of interest

The contributions of Altenburg and Chinapaw were funded by the Netherlands Organization for Health Research and Development (ZonMw; projectnr 91211057). The authors have no other relevant conflicts of interest to disclose.

Acknowledgements

Chinapaw had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Chinapaw and Altenburg. Acquisition of data: Klakk, Møller. Drafting of the manuscript: Chinapaw and Altenburg. Critical revision of the manuscript for important intellectual content: all authors. Statistical analyses: Chinapaw. Obtained funding: Wedderkopp and Andersen. The CHAMPS-study was funded by: The TRYG Foundation, University College Lillebaelt, University of Southern Denmark, The Nordea Foundation, The IMK foundation, The Region of Southern Denmark, The Egmont Foundation, The A.J. Andersen Foundation, The Danish Rheumatism Association, Østifternes Foundation, Brd. Hartmann's Foundation, TEAM Denmark, The Danish Chiropractor Foundation, The Nordic Institute of Chiropractic and Clinical Biomechanics

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Table 1. Characteristics (mean \pm SD, % or median and 25th;75th percentile) of Danish children stratified by weight status^a (n=454)

	Normal weight (n=381)		Overweight (n=73)	
	T1	T2	T1	T2
Age (yrs)	10.3 \pm 0.8		10.1 \pm 0.9	
Gender (boys)	49%		36%*	
Parental education (at least Bachelor degree)	53%		41%	
Puberty (pre-pubertal) ^b	21%		18%*	
BMI (kg/m ²) ^a	16.6 \pm 1.4		21.3 \pm 1.9*	
Counts per minute (average/day)	506 \pm 122	516 \pm 149	465 \pm 105*	449 \pm 109*
MVPA (min/day)	59 \pm 19	61 \pm 23	49 \pm 16*	48 \pm 18*
MVPA in bouts \geq 10 min (min/week)	0 (0;23)	15 (0;37)	0 (0;13)	11 (0;23) *
Number of MVPA bouts per day	0.1 \pm 0.2	0.2 \pm 0.3	0.1 \pm 0.2	0.1 \pm 0.1*
Total sedentary time (min/day)	414 \pm 80	428 \pm 87	426 \pm 101	423 \pm 75
Sedentary time in bouts \geq 10 min (min/day)	196 \pm 79	217 \pm 84	208 \pm 103	217 \pm 73
Number of sedentary bouts per day	10.4 \pm 2.9	11.1 \pm 3.1	10.6 \pm 3.1	11.1 \pm 2.5

^a Body mass index (BMI) was calculated as weight/height² (kg/m²) and the cut-points for overweight proposed by the International Obesity Task Force were used[29].

^b Boys were presented with five pictures and text of Tanner staging for pubic hair development, whereas girls were presented with five pictures and text representing breast development and pubic hair.

* statistically significant difference (p<0.05) between normal weight and overweight children.

Table 2. Cardiometabolic scores (mean \pm SD or median and 25th;75th percentile) at follow-up of Danish children stratified by weight status (N=454)

	Normal weight		Overweight	
Waist circumference (cm)	63.4 \pm 6.0	n=320	76.1 \pm 7.4*	n=59
Systolic blood pressure (mm Hg)	99.0 \pm 7.3	n=381	104.5 \pm 7.9*	n=73
HOMA-IR	1.1 \pm 1.2	n=329	1.5 \pm 0.7*	n=61
Total Cholesterol/HDLC (mm/L)	2.6 \pm 0.6	n=329	3.0 \pm 0.7*	n=61
Triglycerides (mg/L)	54.8 \pm 23.5	n=329	65.3 \pm 27.8*	n=61
Cardiometabolic risk z-score	-0.03 \pm 0.53	n=329	0.67 \pm 0.60*	n=61

HOMA-IR=homeostasis model assessment insulin resistance

* statistically significant difference ($p < 0.05$) between normal weight and overweight children.

Table 3. Associations of sedentary time and moderate to vigorous physical activity (MVPA) with cardiometabolic risk in Danish children (n=454)^a.

	Waist circumference ^a (cm) B (95% CI)	Systolic blood pressure (mm Hg) B (95% CI)	Triglycerides ^b (mg/L) B (95% CI)	Log HOMA-IR ^b B (95% CI)	TC: HDLC ratio ^b B (95% CI)	Cardiometabolic risk score ^b B (95% CI)
Total sedentary time						
• Change in exposure	-1.1 (-2.6;0.5)	-0.8 (-2.2;0.7)	0.2 (-1.1;1.5)	2.3 (0.7;4.0)	0.9 (-0.7;2.6)	0.4 (-0.6;1.4)
• Mean exposure	-1.4 (-2.9;0.02)	-0.4 (-1.7;0.9)	0.7 (-0.5;1.9)	1.0 (-0.5;2.6)	0.5 (-1.0;2.0)	0.0 (-0.9; 0.9)
Sedentary time in bouts						
• Change in exposure	-0.5 (-1.8;0.8)	-0.3 (-1. 5;0.9)	0.2 (-0.9;1.3)	1.4 (-.03;2.8)	1.0 (-0.4;2.4)	0.3 (-0.5;1.2)
• Mean exposure	-0.7 (-1.9;0.6)	0.1 (-1.0;1.3)	0.4 (-0.7;1.4)	0.3 (-1.0;1.7)	0.6 (-0.8;1.9)	0.04 (-0.8;0.8)
MVPA						
• Change in exposure	-3.4 (-7.7;0.8)	-2.0 (-6.0;2.0)	-1.2 (-4.7;2.3)	-6.6 (-11.1;-2.1)	-4.2 (-8.6;0.2)	-3.2 (-5.9;-0.6)
• Mean exposure	-7.3 (-11.3;-3.0)	-2.4 (-6.3;1.5)	-3.6 (-7.1;-0.1)	-7.6 (-12.1;-3.2)	-5.7 (-10.1;-1.2)	-5.2 (-7.8;-2.5)
MVPA in bouts						
• Change in exposure	-14.8 (-34.8;5.2)	-15.1 (-34.7;4.5)	-9.4 (-26.0;7.2)	-11.6 (-33.1;9.9)	-7.5 (-28.5;13.6)	-12.7 (-25.3;0.04)
• Mean exposure	-19.1 (-48.3;10.0)	-29.9 (-55.0;1.1)	-9.0 (-33.1;15.2)	-8.4 (-39.6;22.8)	1.9 (-28.7;32.5)	-13.0 (-31.5;5.5)

^aN=379, ^b N=390

HOMA-IR=homeostasis model assessment insulin resistance, TC:HDLC ratio= total cholesterol: high-density lipoprotein cholesterol ratio, CI=confidence Interval

Boldface type indicates statistical significance P < 0.05.

Outcomes are expressed as standardized z-scores. All models are adjusted for gender, age, parental education, puberty, and schooltype.

Change in exposure is calculated as the score at T2 adjusted for the score at T1 and therefore represents change in exposure adjusted for baseline values. Mean exposure indicates the mean exposure over T1 and T2.

Table 4. Associations of sedentary time and moderate to vigorous physical activity (MVPA) with cardiometabolic risk in Danish children after mutual adjustment for MVPA and sedentary time, respectively (n=454).

	Waist circumference ^a (cm) B (95% CI)	Systolic blood pressure (mm Hg) B (95% CI)	Triglycerides ^b (mg/L) B (95% CI)	Log HOMA-IR ^b B (95% CI)	TC: HDLC ratio ^b B (95% CI)	Cardiometabolic risk score ^b B (95% CI)
Total sedentary time						
• Change in exposure	-3.7 (-5.5;-1.8)	-2.2 (-4.0;-0.3)	-0.8 (-2.0;1.3)	1.0 (-1.1;3.1)	-0.4 (-2.5;1.8)	-0.9 (-2.2;0.4)
• Mean exposure	-5.8 (-7.6;-3.9)	-1.7 (-3.5;0.1)	-0.3 (-1.9;1.3)	1.3 (-3.3;0.8)	-1.5 (-3.5;0.6)	-2.1 (-3.4;-0.9)
Sedentary time in bouts						
• Change in exposure	-1.6 (-3.0;-0.2)	-0.8 (-2.2;0.5)	-0.1 (-1.4;1.1)	0.4 (-1.1;1.9)	0.3 (-1.2;1.9)	-0.4 (-1.3;0.6)
• Mean exposure	-2.6 (-4.0;-1.1)	-0.3 (-1.7;1.0)	-0.3 (-1.5;0.9)	-1.2 (-2.8;0.3)	-0.5 (-2.0;1.1)	-1.1 (-2.0;-0.2)
MVPA						
• Change in exposure	-9.7 (-14.9;-4.6)	-5.8 (-11.0;-0.7)	-1.8 (-6.3;2.7)	-5.0 (-10.7;0.8)	-4.8 (-10.6;0.9)	-4.8 (-8.3;-1.4)
• Mean exposure	-18.4 (-23.8;-13.0)	-5.6 (-10.8;-0.5)	-4.2 (-9.0;0.5)	-10.2 (-16.2;-4.2)	-8.6 (-14.6;-2.6)	-9.4 (-13.0;-5.9)
MVPA in bouts						
• Change in exposure	-19.9 (-40.1;0.3)	-18.2 (-38.1;1.8)	-9.1 (-25.9;7.7)	-6.5 (-28.2;15.2)	-5.5 (-26.8;15.9)	-12.2 (-25.1;0.6)
• Mean exposure	-25.3 (-54.6;4.0)	-29.2 (-57.6;-0.8)	-7.2 (-31.6;17.1)	-5.2 (-36.7;26.3)	3.4 (-27.5;34.3)	-13.3 (-32.0;5.4)

^aN=379, ^b N=390

HOMA-IR=homeostasis model assessment insulin resistance , TC: HDLC ratio= total cholesterol: high-density lipoprotein cholesterol ratio, CI=confidence Interval

Boldface type indicates statistical significance $P < 0.05$.

Outcomes are expressed as standardized z-scores. All models are adjusted for gender, age, parental education, puberty, schooltype, sedentary time by wear time (when MVPA or MVPA in bouts was the outcome), MVPA by wear time (when total sedentary time or sedentary time in bouts was the outcome).

Change in exposure is calculated as the score at T2 adjusted for the score at T1 and therefore represents change in exposure adjusted for baseline values.

Mean exposure indicates the mean exposure over T1 and T2.