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Prospective association of adiposity and cardiorespiratory fitness with cardiovascular risk factors in healthy children

Authors, author's advanced degrees and emails:
Heidi Klakk*,†, PhD student, email: hklakk@health.sdu.dk
Anders Grøntved*, PhD, email: agroentved@health.sdu.dk
Niels Christian Møller*, PhD, email: ncmoller@health.sdu.dk
Malene Heidemann‡ , MD, PhD student, email: msheidemann@dadlnet.dk
Lars Bo Andersen*,§ , Dr.med.scient. email: lboandersen@health.sdu.dk
Niels Wedderkopp *, ‖, MD, PhD, email: nwedderkopp@health.sdu.dk

Author's affiliation:
* Centre of Research in Childhood Health, Department of Sports Science and Clinical Biomechanics, University of Southern Denmark
† University College Lillebaelt, Denmark
‡Hans Christian Andersen Children's Hospital, Odense University Hospital, Denmark
§ Department of Sport Medicine, Norwegian School of Sport Sciences, Oslo, Norway
‖Orthopedic Department, Institute of Regional Health Services Research, University of Southern Denmark, Hospital of Middelfart, Denmark

Address for correspondence
Heidi Klakk
Institute of Sports and Clinical Biomechanics (IOB)
Center of Research in Childhood Health (RICH)
University of Southern Denmark
Campusvej 55, 5230 Odense M
Mob 0045 21356284
Email hklakk@health.sdu.dk

Abstract
Objective: To examine the prospective association of three different measures of adiposity and cardiorespiratory fitness (CRF) with 2-year change in cardiovascular disease (CVD) risk factors in children.
Methods: Two-year longitudinal study in school children aged 7-11 years (n=729). Total body fat (TBF) from DXA, body mass index (BMI), waist circumference (WC), CRF, blood samples and blood pressure were obtained at in 2008 and 2010 in the CHAMPS study-DK.
Results: Greater adiposity at baseline and change in adiposity were associated with increased CVD risk factor levels at follow up. The magnitudes of associations were similar regardless of adiposity measure (TBF%: β 0.30, 95%CI: 0.21 to 0.39, BMI: β 0.24, 95%CI: 0.14 to 0.33, WC: β 0.20, 95%CI: 0.10 to 0.31), and no evidence of non-linear relationships was observed. We found less strong associations of CRF with change in CVD risk factor levels after adjusting for adiposity; however, greater change in CRF was still favorably associated with change in CVD risk factor levels among boys.
Conclusions: Results suggest that any effort to shift the population distribution of adiposity downwards would be valuable for early CVD prevention. The association of CRF with CVD risk factors was largely explained by adiposity, particularly among girls.

Keywords: children, adiposity, cardiorespiratory fitness, cardiovascular risk, CHAMPS study-DK, longitudinal

Prospective association of adiposity and cardiorespiratory fitness with cardiovascular risk factors in healthy children

Klakk H, Grøntved A, Møller NC, Heidemann M, Andersen LB, Wedderkopp N

Introduction

During recent decades worldwide prevalence of obesity has increased substantially. Ten percent of the world's school aged children are estimated to carry excess body fat. In The United States this prevalence is estimated to be more than 30%, in Europe 20% and prevalence increasing by 0.5 to 1% every year (Lobstein et al. 2004). Childhood obesity is associated with a number of immediate cardiovascular health consequences. A recent quantitative assessment of the available cross-sectional evidence suggest unfavorable levels of CVD risk factors among overweight and obese children and adolescents compared with normal weight based on body mass index (BMI) (Friedemann et al. 2012). The evidence from longitudinal studies suggesting that adiposity is associated with CVD risk factors in childhood is, however, more limited (Lawlor et al. 2010; Marcus et al. 2012). In particular, longitudinal studies in young children with assessment that can distinguish fat mass from lean mass are scarce.

Low cardiorespiratory fitness (CRF) is a well-characterized risk factor for increasing levels of CVD risk factors in children and adults (Kodama et al. 2009; Ruiz et al. 2009). Previous observations among adults suggest that maintaining or improving CRF eliminate some of the adverse effects of gains in adiposity on CVD risk (Lee et al. 2011). Whether this is the case among children has been investigated in cross sectional studies (Eisenmann et al. 2007; Jago et al. 2010) and one longitudinal study; the HEALTHY trial (Baranowski et al. 2013; Jago et al. 2013) but to our knowledge this has not been investigated longitudinally in children as young as in the present study.

In this study we examined the prospective association of three different measures of adiposity and CRF with CVD risk factors in a cohort of apparently healthy 7-11-year old Danish children followed over 2 years. Furthermore, we examined the association of change in adiposity and CRF with change in CVD risk factor levels during follow-up.

Materials and Methods

This two-year longitudinal study is nested in the Childhood Health, Activity and Motor Performance School Study in Denmark (CHAMPS study-DK), which is described in detail elsewhere (Wedderkopp et al. 2012). The CHAMPS study-DK was set up to evaluate the effect of increased volume of physical education lessons in public schools on childhood health in general. The study encompasses 10 public schools (n= 1218 children at baseline) located in the Municipality of Svendborg, Denmark.

Ethical approval

All children and parents from the participating schools received information about the study through school meetings and written information. Parents signed informed consent forms for joining the project. Participation was at all times voluntary. Permission to

conduct The CHAMPS study–DK was granted by the Regional Scientific Ethical Committee of Southern Denmark (ID S-20080047).

Study Population
Children from 2nd to 4th grade (n=800, 7.7-12 years) in the CHAMPS study-DK were the population of this study. This sub sample was chosen because only this age range had a whole body DXA scan to provide a direct measure of total body fat percentage (TBF%). Of these 800 children, 739 had a DXA scan at either baseline or follow-up. Of these, 365-438 had complete data on outcome, exposure, and covariates (displayed in Table 1)

Measurements
All measurements were obtained at baseline (2008) and follow-up (2010). Schools and classes were tested in the same order at both time points. Parental education level and birth weight were collected from questionnaires during the first school year.

Adiposity
Body Mass Index (BMI)
Weight was measured to the nearest 0.1 kg on an electronic scale, (Tanita BWB-800S, Tanita Corporation, Tokyo, Japan) wearing light clothes. Height was measured to the nearest 0.5 cm using a portable stadiometer, (SECA 214, Seca Corporation, Hanover, MD). Both anthropometrics were conducted barefoot. BMI was calculated as [weight (kg)/height^2 (m)].

Total Body Fat Percentage (TBF%)
Total body fat mass was measured by Dual Energy X ray Absorptiometry (DXA), (GE Lunar Prodigy, GE Medical Systems, Madison, WI), ENCORE software (version 12.3, Prodigy; Lunar Corp, Madison, WI). The child was instructed to lie still in a supine position wearing under wear, a thin T-shirt, stockings and a blanket for the duration of the DXA scan. All scans were performed by two different operators and analyzed by one of them. The DXA machine was reset every day, following standardized procedures. TBF% was calculated for each participant from the equation: [(TBF (g) x 100)/ weight (g)].

Waist circumference (WC)
WC was measured to the nearest 0.5 centimeter (cm) at umbilicus level after a gentle expiration. Two measurements were undertaken and a consecutive third measure performed if the difference between the first two measurements exceeded one cm. An average of the two nearest measurements was calculated and used in the analyses.
Cardio respiratory fitness (CRF)
CRF was assessed by the Andersen test, a 10 minutes intermittent running test validated against directly measured maximal oxygen uptake (Andersen et al. 2008). Validity (r^2=0.85 compared to VO_{2max} treadmill test) and reliability (r^2=0.86 test/retest) of this field test was tested and described thoroughly for the age group of our cohort before testing (Ahler et al. 2012).
The total distance measured in meters was the test result.

CVD risk factors

Blood samples

Fasting blood samples were obtained between 8.00 and 10.30 in the morning of testing. Samples were kept on ice and handed in to the laboratory within 4 hours, pipetted and centrifuged and kept at -80 degrees Celsius until analyzed. TC, TG, HDL-C and glucose were analyzed by quantitative determination using enzymatic, colorimatic method on Roche/Hitachi cobas c systems. Insulin was analyzed using solid phase enzyme-labeled chemiluminescent immunometric assay.

Total Cholesterol:HDL-C ratio (TC:HDL), was calculated. Homeostasis Assessment Model (HOMA-IR) assessed insulin resistance. The HOMA-IR score was calculated from insulin (μU/ml) x glucose (mmol/l)/22.5 as described by Matthews et al (Matthews et al. 1985). The reliability of HOMA-IR, as a measure of insulin resistance in a large scale and general population, has been shown by Bonora et al (Bonora et al. 2007; Bonora et al. 2000).

Blood pressure

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

Composite risk score

A composite risk score was constructed by summing standardized scores of logHOMA-IR, SBP, logTC:HDL and logTG. A low value of composite-risk score is considered healthier than high values of composite-risk score.

Pubertal Stage

The Tanner pubertal stages self-assessment questionnaire (SAQ) was used to determine pubertal status (Tanner 1962). Boys were presented with five pictures of Tanner staging for pubic hair development, whereas girls were presented with five pictures representing breast development and pubic hair(Duke et al. 1980). Explanatory text in Danish supported the self-assessment. The children were asked to indicate which stage best referred to their own pubertal stage. The procedure took place in a private space with sufficient time to self assess the pubertal stage.

For analysis pubertal stage was dichotomized (Tanner Stage 1 = pre-pubertal, Tanner Stage ≥2 = pubertal) as very few children were classified as Tanner Stage 3-5.

Statistical Analysis

Association of adiposity and CRF with CVD risk factors was examined in multilevel mixed effect regression analyses using the xtmixed procedure in STATA, with school class and school as random effects to comply with the cluster structure of the school-based design. Two models were fitted for each adiposity measure of interest; one with and one without adjusting for CRF level. This was done to be able to estimate the

independent association of CRF and adiposity and to explore if alternative mechanisms could explain the relationships, as both exposures are putative intermediate variables in the causal pathway (i.e. adiposity is an intermediate variable between CRF and CVD risk). Akaikes Information Criteria (AIC) was calculated as a measure of relative quality of the statistical models of predicting CVD risk. In terms of interpretation, the model with the lowest AIC provides the best-fit model. Analyses were adjusted for baseline values of the respective outcome, age, gender, puberty, school type (sports school/normal school), birth weight, and parental educational level. Height and height squared was added to the model, when WC was the explanatory variable of interest. To be able to compare the magnitude of the association based on the adiposity indicators and CRF we standardized these variables before running the analysis.

Deviation from normal distribution of dependent and independent variables of interest was tested. WC, HOMA-IR, TC:HDL ratio and TG, were slightly skewed and log transformed.

Assumption of linearly association between explanatory variables of interest (adiposity and CRF) and outcome of interest (composite score and single factors for CVD risk) was graphically examined. Adding a squared variable of the exposure of interest statistically tested assumption of any curve linearity in the association.

Pearson's correlation and tracking coefficients was calculated by multilevel multivariate regression analysis adjusted for age and gender and random effects.

Effect modification by gender, age, puberty and CRF level at baseline was explored by adding an interaction term between the modifier and primary explanatory variable in the regression (BMI, WC, TBF% and CRF). If the p value of interaction term was ≤ 0.05 an interactions term was added to the model or separate analyses were performed.

Sensitivity analyses were performed imputing missing values on the outcomes and covariates (n=22-189) using chained equations ("mi impute chained" in STATA) including all other covariates (respective outcome at baseline, age, gender, pubertal status, school type, parental educational status, and birth weight) and random effects (indicators for school class and school). Beta coefficients and standard errors (SE) were obtained based on 20 imputed datasets.

All analyses were carried out in STATA (version12.1) with $\alpha=0.05$ (two-sided).

Results

The characteristics of the cohort comparing the children with complete data (n=365) to those with non-complete data are shown in Table 1. Children with complete data were slightly higher, had better CRF, lower WC, TBF% and BMI and more children had entered puberty than those with non-complete data. For all other covariates and outcomes there were no significant differences between groups, see Table 1.

The three measures of adiposity was strongly correlated to each other (age and gender adjusted correlation coefficient: TBF% and BMI 0.80, TBF% and WC 0.79, BMI and WC 0.89, all p values <0.001) and strongly correlated over time (age and gender adjusted tracking coefficients; TBF%: 0.91, BMI 0.93, WC 0.84, all p values <0.001).

Baseline adiposity by all three measures was independently and positively associated to increased composite CVD risk score after 2 years. TBF% was the strongest predictor of increased single and composite CVD risk factors. The statistical model with TBF% included explained more of the variation of composite risk score than BMI, WC and CRF (AIC 811.4 compared to 825.5, 827.5 and 811.5 respectively) even more so when the model including TBF% was adjusted for CRF as well (AIC 782.5). Estimates for the association of adiposity with CVD risk only changed slightly downwards when adjusted for CRF at baseline (see Table 2). We did not find statistical evidence that any of these associations were modified by gender ($p>0.05$ for interaction). Including a quadratic term of adiposity or fitness, gave no evidence of a curve-linear relationship between adiposity and CVD risk factors (BMI^2 $p=0.56$, $TBF\%^2$ $p=0.53$, WC^2 $p=0.66$). Graphically evaluating the shape of associations of the three adiposity measures and CRF to CVD risk also gave no reason to reject assumption of linearity.

Baseline CRF was significantly and inversely associated with composite CVD risk factor score (β -0.12 SD, 95% CI: -0.21 to -0.02). When the model was adjusted for baseline adiposity the association attenuated and became non-significant (see Table 2). Stability of CRF over time was lower than for adiposity (age and gender adjusted tracking coefficient: 0.67)

Associations of adiposity indicators and CRF to single risk factors are shown in table 2. TBF% was the strongest predictor for all single risk factors, BMI was very similar whereas WC only significantly predicted increase in HOMA-IR and TG, but not SBP and TC:HDL. Cardiorespiratory fitness was inversely associated with HOMA-IR, but not with the other three single risk factors.

Sensitivity analysis comprising n=739 did not change estimates of association for neither cardiorespiratory fitness nor adiposity indicators (see supplementary Table 1).

We also examined the association of change in adiposity and CRF with change in CVD risk factor levels. Changes in adiposity (Δ TBF%, ΔBMI or ΔWC) were all similarly associated with change in CVD risk factor levels (Table 3). We observed that the associations of changes in WC and changes in CRF with the composite CVD risk score were stronger in boys compared with girls (WC: β 0.21, $p=0.01$ for interaction, CRF: β -0.19, $p=0.02$ for interaction). Associations were in the same direction but increasing WC for boys had stronger association to CVD risk than for girls (β 0.36 SD, 95% CI: 0.22 to 0.50 compared to β 0.18 SD, 95% CI: 0.08 to 0.29) and for CRF the gender difference was likewise a stronger effect of increasing CRF level for boys than girls (β -0.27 SD, 95% CI: -0.39 to -0.14 compared to β -0.14 SD, 95% CI: -0.27 to -0.01). The association was independent of change in adiposity for boys, however, for girls the association was non-significant after this additional adjustment.
We found no indication of interaction between change in adiposity and change in CRF on the change in CVD risk factor levels (p values between 0.10 and 0.97). Also no interaction was found between these indicators as continuous variables (p values between 0.31 and 0.72) indicating an additive and not multiplicative effect of status or change in CRF to adiposity status or change.

Very few children in our sample changed weight category from normal weight to OW/OB and vice versa. Thus we were not able to statistically test the association of changing weight category. We did however estimate the association of CVD risk for the children defined OW/OB according to international standards (Cole et al. 2000) at baseline having the normal weight children as reference group. These estimates suggest that OW/OB children had worse CVD risk factor levels after two years. This association was attenuated after adjustment for CRF but still significantly different from the normal weight children except from association with triglyceride levels, which became non-significant adjusting for CRF (Table 4).

Discussion

In this longitudinal study greater adiposity at baseline and increase in adiposity, based on TBF%, BMI, or WC, was independently associated with unfavorable 2-year changes in levels of CVD risk factors in healthy school children aged 7-11 years. TBF% was the strongest predictor of composite- and single CVD risk factor change and we found no evidence of non-linear relationships. This suggests that lowering adiposity even inside the range of normal weight could be favorable for prevention of raised levels of CVD risk factors in healthy primary school children. Furthermore, we found that the association of CRF with change in CVD risk factor levels to some extent was explained by concomitant change in adiposity, particularly in girls. Our analysis suggested an additive effect of adiposity and CRF, indicating that in healthy primary school children lowering adiposity is beneficial regardless of CRF level and vice versa.

The findings from our study are supported by two other recent longitudinal studies in children and adolescents. In The ALSPAC study, a longitudinal study of more than 5000 children from the U.K. aged 9 to 12 at baseline, Lawlor et al (Lawlor, Benfield 2010) examined the association of adiposity by the same three indicators of adiposity as used in our study and reported very similar results on associations of adiposity and CVD risk. In parallel to our findings they found that BMI, WC and TBF% were highly correlated, and were associated with change in CVD risk factor levels with similar magnitudes. Furthermore, Marcus et al(Marcus, Foster 2012) reported longitudinal results from the HEALTHY trial showing that 2.5-year changes in BMI was strongly associated with changes in single CVD risk factors in U.S. children from 6^{th} to 8^{th} grade (11 to 13 years). In addition, based on the same cohort Jago et al. (Jago et al. 2013) reported that WC did not add much to the association compared to BMI, except from being better associated to fasting glucose. This is in line with our findings where an increase in WC was only significantly associated to increased HOMA-IR and TG whereas TBF% and BMI was strongly associated to all single risk factors. The HEALTHY trial did not, however, provide direct measures of total body fat by DXA. Hence, our results extend the evidence of the association of adiposity and CVD risk to even younger children than the above-mentioned studies, as our cohort was 2^{th} to 4^{th} grade (7.4 to 11.6 years) followed for two years.

We did not find any reason to reject linearity in the association of adiposity CVD risk. This is supported by the previous mentioned report from the ALSPAC cohort (Falaschetti et al. 2010) and results from a large longitudinal cohort study by Baker et al (Baker et al.

2007). They examined the association of BMI and later coronary heart disease in a large cohort of Danish schoolchildren (276.835 children followed up 25 years later). They concluded that the association of childhood BMI (7 through 13 years of age) to later coronary heart disease was linear across the entire BMI distribution.

Because CRF level to a large extent is explained by long-term engagement in physical activity, it is likely that adiposity is on the causal pathway between CRF and CVD risk. Our results suggested that the association of CRF with CVD risk was largely explained by adiposity and indicate that this is a key mechanism that explains the inverse association of CRF with CVD risk factors in primary school children. This finding is in line with other longitudinal studies in children (Jago, Drews 2013; Wang et al. 2011), but not consistent with cross sectional studies (Ekelund et al. 2007; Jago, Drews 2010) where CRF has been found to be associated with CVD risk independent of adiposity. The attenuation of the association of CRF to CVD is not in line with results in the adult population (Lee et al. 2010). This might suggest that the benefits of CRF are not independent of age, and therefore high fitness becomes more important in youth and adulthood. This speculation is supported by a study of Ondrak *et al* (Ondrak et al. 2007). They showed that amongst more than n=1800, 8-16 years old children, association of adiposity to CVD risk declined with age where as associations of CRF tended to increase with age.

The finding that greater change in CRF was observed to be more beneficial for boys than girls has to our knowledge not been shown in other studies. Also, results suggest that greater central adiposity has greater effects of increasing CVD risk factor levels for boys, although girls increased their WC significantly more than boys. An increase in WC is expected during two years in this age group and a plausible biological explanation to the observed sex-specific associations could be the discordant physiological maturation that occurs between boys and girls.

Strength and Limitations

The strengths of this study are the longitudinal design and the different indicators of adiposity including a direct measure TBF% by DXA. Furthermore, the detailed collection of covariates including CRF allowed for adjustment for several potential confounders. A number of possible limitations should be considered. The cohort consists of a majority of healthy, normal weight Caucasian children. This may limit the generalizability of our results to other ethnic groups. Our measurement of CRF was obtained by an indirect measure. Thus, even though we documented high agreement between the field-test and directly measured VO_2-max, some measurement error will remain and it is difficult to compare estimates of CRF and adiposity as these will be affected by different degrees of measurement error. The composite risk score used was constructed by simply summing up equally weighted single risk factors and their predicate validity in childhood for clinical health outcomes in adulthood remains unknown. Furthermore, the high attrition rate may have affected the generalizability of our findings, but since associations in imputed and not-imputed samples were fairly similar, we have confidence that the results are not explained by selection bias. Finally, as our study was observational, it is prone to unknown and residual confounding.

In summary, we found that greater adiposity at baseline and increasing change in adiposity status from baseline to follow-up were independently and linearly associated with unfavorable 2-year change in CVD risk factor levels in Danish primary school children. We observed less strong prospective associations of CRF with change in CVD risk factor levels after adjustment for adiposity; however, change in CRF was independently associated with change in CVD risk factor among boys. The magnitudes of associations of baseline values of adiposity with CVD risk were similar regardless of which indicator of adiposity that was used.

Perspectives
Increasing children's CRF level will attenuate, but not eliminate, the adverse risk of adiposity. Any effort to decelerate or downward shift the population distribution of adiposity would be valuable for early CVD prevention in primary school children. High levels of sports and physical activity in childhood are likely to be the key mechanism to change both factors simultaneously.

Conflicts of interest statement:
The authors have no financial or other conflict of interest to declare.

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Table legends:

Table 1: Baseline characteristics of participants followed for 2 years and those lost to follow up/missing data.

[a] P is for difference between individuals with complete data and individuals with missing data or that were lost to follow-up.

[b] Defined according to International Obesity Task Force (IOTF) criteria (Cole et al 2000)

[c]Below /above bachelor degree

CRF = cardiorespiratory fitness, TBF=Total Body Fat, BMI= Body Mass Index, WC=Waist Circumference, HOMA-IR= Homeostasis Assessment Model for insulin resistance, SBP=systolic blood pressure, TC:HDL= Total Cholesterol/High Density Lipoprotein, TG= triglycerides.

N=729 children measured in Denmark 2008 and 2010

Table 2: Association of adiposity and Cardiorespiratory Fitness to CVD composite score and single risk factors

[a]Model 1:analyses were adjusted for baseline values of risk, age, gender, school type, pubertal status, birth weight, and parental educational level (associations of WC also adjusted for height and height^2)

[b] Model 2: additionally adjusted for fitness (or TBF% when fitness was the exposure of interest)

[c] β coefficient represents change in risk factor (expressed in SD) per 1 SD in exposure(adiposity or fitness). The value of 1 SD is displayed in parenthesis after each outcome/exposure.

BMI= Body Mass Index, HOMA-IR= Homeostasis Assessment Model for insulin resistance, BP=blood pressure, TC:HDL= Total Cholesterol/High Density Lipoprotein, TG= triglycerides.

Numbers in parenthesis after risk factor and exposure is the values of 1 SD of the variable.

N=349-445 children measured in Denmark 2008 and 2010

Table 3: Associations of change in adiposity and fitness during 2 school years on Composite risk score (zscore)

All analysis adjusted for baseline value of adiposity, age, gender, birth weight, parental educational level and puberty status at baseline.

a adjusted for baseline values of adiposity and CRF and delta values for both

[b] additionally adjusted for height and height^2

[c] adjusted for baseline values of CRF and not of adiposity

Δz = standardized delta value of exposure, TBF%=Total Body Fat Percentage, BMI= Body Mass Index, WC=Waist Circumference, CRF = cardiorespiratory fitness, adj=adjusted

N=343-359 children measured in Denmark 2008 and 2010

Table 4: Association of weight status at baseline with change in CVD risk factor levels. Estimates are for the OW/OB children at baseline with normal weight children being the reference group.

a Model 1:analyses were adjusted for baseline values of risk, age, gender, school type, pubertal status, birth weight, and parental educational level

[b] Model 2: additionally adjusted for fitness (CRF)

[c] OW/OB= overweight and obesity categories defined according to International Obesity Task Force (IOTF) criteria (Cole et al 2000, (Cole, Bellizzi 2000)) collapsed to one category

IOTF= International Obesity Task Force HOMA-IR= Homeostasis Assessment Model for insulin resistance, BP=blood pressure, TC:HDL= Total Cholesterol/High Density Lipoprotein, TG= triglycerides.

Numbers in parenthesis after risk factor is the value of 1 SD for the respective outcome

N=387-445 children measured in Denmark 2008 and 2010

Table 1: Baseline Characteristics of Participants followed for 2 years and those lost to follow up/missing data

[a] P is for difference between individuals with complete data and individuals with missing

Variable Mean (SD)	n	Individuals with complete data n=365	n	Individuals with missing data or that were lost to follow up (n=374-185)	P[a]
Age (years) Range (years)	365	9.4(0.8) 7.7 to 11.4	374	9.3(0.9) 7.4 to 11.6	0.60
Gender Girls/boys n(%)	365	187(51)/178(49)	374	197(53)/177(47)	0.70
Height (cm)	365	138.8 (7.4)	345	137.0 (7.6)	0.01
Weight (kg)	365	32.3(6.2)	345	32.0(6.4)	0.90
BMI (point)	365	16.7(2.0)	345	16.9(2.3)	0.05
TBF(%)	365	19.9 (7.5)	352	21.2(8.7)	0.02
WC (cm)	365	59.8(6.4)	344	61(7.3)	0.01
CRF (m)	351	938 (97.8)	323	916.6(105.8)	0.02
HOMA-IR	365	0.85 (0.5)	256	0.89 (0.7)	0.60
TG (mmol/L)	365	0.66 (0.3)	256	0.64(0.3)	0.90
TC:HDL	365	2.7 (0.6)	256	2.8 (0.8)	0.05
SBP (mmHg)	365	103.3(7.0)	310	102.1 (8.5)	0.50
Composite Risk score (zscore)	365	0.19 (0.9)	220	0.10 (1.1)	0.80
Pre-pubertal/pubertal N (%)	365	215(59)/150(41)	316	217(68)/99(32)	0.01
School type Sports/normal – N/%	365	210(57)/155(43)	374	208(56)/166(44)	0.70
Overweight[b] N(%)	365	37 (10)	345	45 (13)	0.25
Obese[†] N(%)		3 (1)		8 (2)	0.13
Parental Educational level [c] N %	365	175/190 48/52	286	146/140 51/49	0.44
Birth Weight (kg)	365	3.5(0.7)	185	3.6(0.7)	0.40

data or that were lost to follow-up.

[b]Defined according to International Obesity Task Force (IOTF) criteria (Cole et al 2000)

[c]Below /above bachelor degree

CRF = cardiorespiratory fitness, TBF=Total Body Fat, BMI= Body Mass Index, WC=Waist Circumference, HOMA-IR= Homeostasis Assessment Model for insulin resistance, SBP=systolic blood pressure, TC:HDL= Total Cholesterol/High Density Lipoprotein, TG= triglycerides.
N=729 children measured in Denmark 2008 and 2010

Table 2: Association of adiposity and Cardiorespiratory Fitness to CVD composite score and single risk factors

Outcome (1 SD)	Model 1[a]				Model 2[b]			
	N	β[c]	(95% CI)	P	N	β[c]	95% CI	P
			Total Body Fat% (SD: 7.5 %)					
Composite risk score	365	0.30	(0.21 to 0.39)	<0.001	349	0.28	(0.17 to 0.38)	<0.001
HOMA-IR (0.85)	386	0.36	(0.25 to 0.46)	<0.001	368	0.32	(0.20 to 0.44)	<0.001
Systolic BP(7.0 mmHg)	443	0.18	(0.09 to 0.46)	<0.001	442	0.20	(0.11 to 0.30)	<0.001
TC:HDL (0.6)	386	0.10	(0.04 to 0.17)	0.002	368	0.09	(0.01 to 0.16)	0.02
TG (0.3mmol/L)	386	0.19	(0.09 to 0.28)	<0.001	368	0.17	(0.06 to 0.27)	0.003
			BMI (SD:2.0 points)					
Composite risk score	367	0.24	(0.15 to 0.33)	<0.001	351	0.21	(0.11 to 0.30)	<0.001
HOMA-IR (0.85)	387	0.27	(0.18 to 0.37)	<0.001	370	0.23	(0.12 to 0.34)	<0.001
Systolic BP (7.0 mmHg)	445	0.15	(0.07 to 0.23)	<0.001	424	0.16	(0.07 to 0.25)	<0.001
TC:HDL(0.6)	387	0.09	(0.03 to 0.15)	0.003	370	0.07	(0.01 to 0.14)	0.04

TG (0.3 mmol/L)	387	0.15	(0.06 to 0.24)	0.001	370	0.12	(0.03 to 0.22)	0.01
			Waist Circumference (SD:6.4 cm)					
Composite risk score	367	0.20	(0.10 to 0.31)	<0.001	351	0.16	(0.05 to 0.28)	0.01
HOMA-IR (0.85)	386	0.27	(0.16 to 0.39)	<0.001	370	0.22	(0.09 to 0.35)	0.001
Systolic BP (7.0 mmHg)	445	0.08	(-0.01 to 0.17)	0.09	424	0.09	(-0.01 to 0.20)	0.08
TC:HDL (0.6)	386	0.07	(-0.008 to 0.14)	0.08	370	0.04	(-0.05 to 0.12)	0.38
TG (0.3mmol/L)	386	0.16	(0.06 to 0.27)	0.003	370	0.15	(0.03 to 0.26)	0.02
			Cardiorespiratory fitness (SD:97.8 m)					
Composite risk score	351	-0.12	(-0.21 to -0.02)	0.02	349	0.009	(-0.11 to 0.09)	0.87
HOMA-IR (0.85)	370	-0.16	(-0.27 to -0.05)	0.05	368	-0.03	(-0.14 to 0.09)	0.66
Systolic BP (7.0 mmHg)	424	-0.05	(-0.14 to 0.04)	0.30	422	0.05	(-0.05 to 0.15)	0.30
TC:HDL (0.6)	370	-0.06	(-0.12 to 0.01)	0.06	368	-0.02	(-0.10 to 0.06)	0.60
TG (0.3mmol/L)	370	-0.09	(-0.19 to 0.01)	0.09	368	-0.02	(-0.13 to 0.10)	0.77

[a] Model 1:analyses were adjusted for baseline values of risk, age, gender, school type, pubertal status, birth weight, and parental educational level (associations of WC also adjusted for height and height^2)

[b] Model 2: additionally adjusted for fitness (or TBF% when fitness was the exposure of interest)
[c] β coefficient represents change in risk factor (expressed in SD) per 1 SD in exposure(adiposity or fitness). The value of 1 SD is displayed in parenthesis after each outcome/exposure.
BMI= Body Mass Index, HOMA-IR= Homeostasis Assessment Model for insulin resistance, BP=blood pressure, TC:HDL= Total Cholesterol/High Density Lipoprotein, TG= triglycerides.
Numbers in parenthesis after risk factor and exposure is the values of 1 SD of the variable
N=349-445 children measured in Denmark 2008 and 2010

Table 3: Associations of change in adiposity and CRF during 2 school years on Composite risk score (SD)

Variable of interest	N	β coefficient	95% CI	p- value
Δ zTBF %	359	0.14	0.06 to 0.22	0.001
+adj for Δ zCRF[a]	336	0.12	0.03 to 0.21	0.01
Δ zBMI	359	0.30	0.22 to 0.37	<0.001
+adj for Δ zCRF[a]	335	0.29	0.21 to 0.38	<0.001
Δ zWC[b]	359	0.27	0.19 to 0.35	<0.001
-girls	183	0.18	0.08 to 0.29	0.001
-boys	176	0.36	0.22 to 0.50	<0.001
+adj for Δ zCRF[a]	335	0.26	0.17 to 0.35	<0.001
-girls	167	0.16	0.05 to 0.28	0.004
-boys	168	0.30	0.17 to 0.43	<0.001
Δ zCRF[c]	343	-0.20	-0.29 to -0.10	<0.001
- girls	173	-0.14	-0.27 to -0.01	0.04
- boys	170	- 0.27	- 0.39 to -0.14	<0.001
+adj for Δ zTBF%[a]	336	-0.08	-0.18 to 0.01	0.08
- girls	168	-0.07	-0.20 to 0.07	0.35
- boys	168	-0.17	-0.31 to -0.03	0.02

All analysis adjusted for baseline value of adiposity, age, gender, birth weight, parental educational level and puberty status at baseline.

[a] adjusted for baseline values of adiposity and CRF and delta values for both

[b] additionally adjusted for height and height^2

[c] adjusted for baseline values of CRF and not of adiposity

Δz = standardized delta value of exposure, TBF%=Total Body Fat Percentage, BMI= Body Mass Index, WC=Waist Circumference, CRF = cardiorespiratory fitness, adj=adjusted

N=343-359 children measured in Denmark 2008 and 2010

Table 4: Association of weight status at baseline with change in CVD risk factor levels. Estimates are for the OW/OB children at baseline with normal weight children being the reference group.

Outcome (1 SD)	Model 1[a]				Model 2[b,]			
	NW/OW-OB	β	(95% CI)	P	N	β	95% CI	P
	Being OW/OB defined by IOTF cut points[c]							
Composite risk score	327/40	0.46	(0.21 to 0.72)	<0.001	313/38	0.32	(0.04 to 0.60)	0.02
HOMA-IR (0.85)	345/42	0.56	(0.27 to 0.84)	<0.001	330/40	0.39	(0.08to 0.70)	0.01
Systolic BP(7.0 mmHg)	391/54	0.27	(0.04 to 0.49)	0.02	373/51	0.28	(0.04 to 0.53)	0.03
TC:HDL (0.6)	345/42	0.19	(0.01 to 0.36)	0.04	330/40	0.09	(0.01 to 0.16)	0.02
TG (0.3mmol/L)	345/42	0.34	(0.08 to 0.60)	0.01	330/40	0.21	(-0.07 to 0.50)	0.14

[a] Model 1:analyses were adjusted for baseline values of risk, age, gender, school type, pubertal status, birth weight, and parental educational level

[b] Model 2: additionally adjusted for fitness (CRF)

[c] OW/OB= overweight and obesity categories defined according to International Obesity Task Force (IOTF) criteria (Cole et al 2000, (Cole, Bellizzi 2000)) collapsed to one category

IOTF= International Obesity Task Force HOMA-IR= Homeostasis Assessment Model for insulin resistance, BP=blood pressure, TC:HDL= Total Cholesterol/High Density Lipoprotein, TG= triglycerides.

Numbers in parenthesis after risk factor is the value of 1 SD for the respective outcome

N=387-445 children measured in Denmark 2008 and 2010

Table legend:

Supplementary table 1: Associations of adiposity indicators and cardiorespiratory fitness with composite CVD risk factor score from multiple imputation analyses.

Missing values on the outcomes and covariates (n=22-189) were imputed using chained equations ("mi impute chained" in STATA) including all other covariates (respective outcome at baseline, age, gender, pubertal status, school type, parental educational status, and birth weight) and random effects (indicators for school class and school). Beta coefficients and standard errors (SE) were obtained based on 20 imputed datasets.

z = standardized value of TBF%=Total Body Fat Percentage, BMI= Body Mass Index, WC=Waist Circumference, CRF= cardiorespiratory fitness.

[a] adjusted for adiposity (TBF%) when CRF is the exposure

Supplementary table 1: Associations of adiposity indicators and cardiorespiratory fitness with composite CVD risk factor score from multiple imputation analyses.

Exposure	Not adjusted for CRF[a]		Adjusted for CRF[a]	
	Beta (95% CI)	p	Beta (95% CI)	p
zTBF%	0.28 (0.20 ; 0.35)	<0.001	0.28 (0.19 ; 0.37)	<0.001
zBMI	0.22 (0.16 ; 0.29)	<0.001	0.20 (0.11 ; 0.28)	<0.001
zWC	0.24 (0.16 ; 0.31)	<0.001	0.21 (0.13 ; 0.29)	<0.001
zCRF	-0.12 (-0.21 ; -0.02)	0.014	0.01 (-0.08 ; 0.10)	0.88

Missing values on the outcomes and covariates (n=22-189) were imputed using chained equations ("mi impute chained" in STATA) including all other covariates (respective outcome at baseline, age, gender, pubertal status, school type, parental educational status, and birth weight) and random effects (indicators for school class and school). Beta coefficients and standard errors (SE) were obtained based on 20 imputed datasets.

z = standardized value of TBF%=Total Body Fat Percentage, BMI= Body Mass Index, WC=Waist Circumference, CRF= cardiorespiratory fitness.

[a]adjusted for adiposity (TBF%) when CRF is the exposure