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TITLE PAGE

Bicycling to school improves the cardiometabolic risk factor profile: a randomised controlled trial

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ABSTRACT

Objectives:

To investigate whether bicycling to school improve cardiometabolic risk factor profile and cardiorespiratory fitness among children.

Design:

Prospective, blinded, randomised controlled trial.

Setting:

Single centre study in Odense, Denmark.

Participants:

43 children previously not bicycling to school were randomly allocated to control group (n=20) (i.e. no change in lifestyle) or intervention group (i.e. bicycling to school) (n=23).

Primary and secondary outcome measures:

Change in cardiometabolic risk factor score and change in cardiorespiratory fitness.

Results:

All participants measured at baseline returned at follow-up. Based upon intention to treat analyses, clustering of cardiometabolic risk factors was lowered by 0.58 SD (95% CI -1.03 to -0.14, p= 0.012) in the bicycling group compared to the control group. Cardiorespiratory fitness (L $O_2 \cdot min^{-1}$) per se did not increase significantly more in the intervention than in the control group (beta = 0.0337, 95% CI -0.06 to 0.12, p=0.458).

Conclusions:

Bicycling to school counteracted a clustering of cardiometabolic risk factors and should thus be recognized as potential prevention of type 2 diabetes mellitus and cardiovascular disease. The intervention did, however, not elicit a larger increase in cardiorespiratory fitness in the intervention group as compared to the control group.

Trial registration:

Registered at www.clinicaltrials.gov (NCT01236222)

ARTICLE SUMMARY

Article focus

- Intervention studies investigating whether bicycling to school in fact cause improved cardiometabolic health have not yet been carried out.
- The aim of this study was to investigate whether bicycling to school cause an increase in cardiorespiratory fitness and induce improvement of clustering of cardiometabolic risk factors

Key messages

- This study is the first study to document that bicycling to school had positive effect on clustering of cardiometabolic risk factors in children and thus in the prevention of diabetes and cardiovascular disease.
- The result from this study suggests investment in infrastructure and promotion of bicycling to school in general.

Strengths and Limitations

- The major strength of this study was the randomised design which through elimination of bias allowed for investigation of a causal relationship between bicycling to school and health outcomes.
- Relatively weak statistical power and impossibility of blindness of the treatment.

INTRODUCTION

The metabolic syndrome (MS) is the concurrence of multiple risk factors associated with cardiovascular disease and type 2 diabetes mellitus. Prevalence is between 20-30% of the adult population in most countries[1], and a rising incidence in children and adolescents[2], indicates that the condition represents a major threat to global public health. Exposure of the MS confers a doubled risk of incident cardiovascular event and death[3] and up to 5-times higher risk of developing type 2 diabetes mellitus in adults[4]. Furthermore, since clustering of cardiometabolic risk factors track from childhood to adulthood[5] early prevention of the MS is important in order to prevent occurrence of disease later in life.

In children and adolescents physical activity has the potential to prevent a clustering of risk factors[6], underpinned by the notion that an increase of daily moderate physical activity by 10-20% is associated with 33% lower risk of having the MS[7].

Active commuting such as walking and bicycling to school might be important contributors of preventive physical activity. Observational studies indicate that bicycling to school is beneficial to health since higher cardiorespiratory fitness[8] and lower BMI[9] have been observed in children bicycling to school compared to non-cyclists. If bicycling to school is generally acknowledged as a time-efficient and feasible form of daily physical activity it could potentially target the entire population. From a public health perspective this would be very appealing considering that the greatest health benefits are achieved in the least active individuals [10].

There seems to be wide-ranging scepticism about the opportunity to bicycle to school in industrialized societies. The level of concern, however seems often to be negatively associated with the prevalence of bicycling to school, which in Denmark for instance is about 60% among adolescents [8,9]whereas in the UK approximately 2% bicycle to school [11].

Intervention studies investigating whether bicycling to school in fact cause improved cardiometabolic health have not yet been carried out. One reason for this could be that very few countries have an infra-structure allowing safe commuting by bike to school. Another reason could be that interventions often include change in the built environment in order to provide safe routes and this is difficult to control in a rigid study design.

The evidence linking bicycle commuting with cardiometabolic health in children is still limited. Hence, the aim of this study is to investigate, in a randomised trial, whether bicycling to school cause an increase in cardiorespiratory fitness and induce improvement of clustering of cardiometabolic risk factors.

METHODS

Study participants

This study took place in Denmark in the municipality of Odense during spring 2011. Participants were recruited through invitation letters sent to 36 elementary public schools in the municipality of Odense. Investigators then visited 10 of these schools (approximately 92 classes) and presented the project. Additionally, the project was presented online and in a radio spot. Participants were included if they had not bicycled regularly to and from school for at least 3 months prior to the intervention, and if willing to be randomised to one of the two study groups (i.e. control group or bicycling group). Reasons for exclusion were not having a bike, and/or affirming less than one km between home and school. One hundred and eighty-nine children volunteered to participate in the study. One hundred and thirty-one of these, however, claimed that they bicycled regularly to school, and four withdrew from the study due to a changed family situation. Fifty-four eligible children were subsequently randomised to either control or intervention. After randomisation six and five participants from control and intervention group, respectively, withdrew since they did not accept being randomized, leaving 43 participants in the study (Figure 1). Written informed consent was obtained from the child's parent or legal guardian after they were given a detailed written explanation of the aims of the study, possible hazards, discomfort, and inconvenience, and the option to withdraw at any time. The study was approved by the regional Ethics Committee (S-20100009) and registered at clinicaltrials.gov (NCT01236222) with the purpose of investigating the effect of bicycling to school on the metabolic syndrome and aerobic fitness.

Study design and intervention

Participants were randomly assigned to a control group (i.e. no change in lifestyle) or to an experimental group (i.e. commuter bicycling). A permuted random block size (2, 4 and 6) randomisation (www.randomization.com) was used in order to reduce the chances of the assignment schedule being seen by those responsible for recruitment of participants[12]. Twenty participants were allocated to the control group and 23 participants to the bicycling group. All measurements were repeated at the conclusion of the 8-week intervention program. There were no restrictions beside the mode of transportation to school. The study period comprised one week of national holiday and additionally two bank holidays where no children attended school. Matching baseline test date with follow-up test was strived for, but logistically not possible for all participants. This has implications on the amount of exposure the intervention group was able to accumulate. Participants could maximally accumulate between 56 and 74 trips to and from school during the study period. All measurements at baseline and follow-up were conducted by personal blinded to group allocation. Children were picked up and returned to their home

addresses when scheduled for baseline and follow-up tests.

Measurements

Height was measured by a stadiometer (SECA, Hamburg, Germany), children wearing socks or bare feet and weight was assessed by a 0.1 kg precision scale (Soehnle Professional, Murrhardt, Germany) wearing only shorts and t-shirts. Skinfold thickness was measured on the left side with a Harpenden caliper (John Bull, British Indicators Ltd., West Sussex, England) at the biceps, triceps, subcapsular, and suprailiac sites. Overweight/obesity status was defined according to age- and gender specific published cut-points for BMI[13].

Systolic and diastolic blood pressure was measured by a sphygmomanometer (DINAMAP ProCare 100, General electric, USA). At least five measurements were made on the left arm with two min interval and the mean of the final three measurements were used as systolic and diastolic pressure.

All Children were instructed to fast overnight (>8 hours), and only allowed to drink water until the blood sample was drawn. Blood was drawn from the right fossa cubitus by a biomedical laboratory scientist. If a participant had not been fasting or if he/she had experienced any illness during the last week, a new test day was scheduled for that child. Samples were analysed for: Glucose, insulin, cholesterol, triglyceride. Insulin resistance was estimated according to the homoeostasis model assessment (HOMA) as the product of fasting glucose (mmol/L) and insulin (µU/mL) divided by the constant 22.5[14]. Breakfast was served on site subsequent of blood sampling. All analysis of the blood specimens were conducted at Department of Clinical Biochemistry and Pharmacology, Odense Universityhospital

Aerobic fitness ($\dot{V}O_2$peak) was determined in a progressive bicycle test with on an electronically braked ergometer (Monark 839 Ergomedic, Varberg, Sweden). Pulmonary gas exchange was measured with a metabolic cart (Amis2001, Innovision, Denmark) with 15 seconds epoch, and the highest value within 30 seconds was regarded as a maximal if RER was >= 0.99 or maximal HR was >=185 beats/min, and the test leader judged the participant to show signs of intense effort (e.g. facial flushing or difficulties in keeping up the pedal frequency)[15]. Heart rate (HR) was measured with a HRM (Polar RS800CX, Kempele, Finland) with epoch set to 1 sec. Children were thoroughly explained the purpose of the test and that it would require a maximal effort. After a warm-up period of 5 minutes at 40W, work-load was increased 40W every 2 minutes until volitional exertion. Verbal encouragement was given throughout the test and recommended pedaling cadence was 60-80 rpm.

Children completed a general questionnaire regarding transportation to school, sports-habits, and general quality of life. At follow up all children marked their route to school using a web-based map tool (www.loebererute.dk).

All children had their bike equipped with an odometer on average two weeks before baseline measurement. The odometers were individually calibrated in accordance to wheel circumference. Odometer levels were self-reported, by participants in both groups, every Sunday until tested at follow-up by means of a commercial SMS system (SMS-Track ApS). Participants who did not answer Sunday were contacted Monday. Malfunctioning odometers were replaced within a few days.

Both groups were instructed to report daily mode of school transportation on a custom made transport diary. Total mileage of school related bicycling during the study was calculated from the distance to school (web route assessment) times the number of trips to/from school (transport diary).

Field measurent of intensity of bicycling to school was carried out midway (five weeks after baseline). Participants in the intervention group received a GPS (Qstarz BT-Q1300S, Qstarz International Inc., Taiwan) and a heart rate monitor (HRM) (PolarTeam^2, Polar, Kempele, Finland) and were instructed to wear the devices during one day. Both GPS and HRM were set at an epoch of 5 second. All GPS data were transferred to commercial software (Travel recorder v. 5.0) and corrected for drift using manufacturer software. The GPS was the reference for all HRM data. Mean HR of school transportation, if verified by the diary, was calculated as the mean of the measurements from the first data point when the child exceeded a speed of 5 km/h when leaving home, to the last data point above 5 km/h when the child arrived at school. Both compliant and non-compliant participants were included in the determination of commuter bicycling intensity since the primary outcome is based on intention to treat (ITT) analyses.

Physical activity beyond bicycling was assessed with an accelerometer (Actigraph GT3X, Manufacturing Technology Inc, Fort Walton Beach, FL, USA). Physical activity was monitored for 7 consecutive days from Monday to Sunday at baseline and midway (five weeks after baseline). Instruments were attached at the hip, and counts were sampled every two seconds. Data reduction was performed with customised software (Propero, Odense, Denmark). Only data from the vertical axis was included in the data analyses. Criteria for a successful recording were a minimum of 3 days of 10 h recording per day. Time periods of at least 30 consecutive minutes of zero counts were deemed to represent periods when the monitor was not worn and thus disregarded. Cut points for intensity levels were based on the Freedson/Trost equation[16]. Since cutpoints for physical activity intensity are specifically designed for 1 min epoch these were divided by 30. The average time the accelerometer was worn was 13.5 and 13.6 h per day at baseline and follow up, respectively, and the number of minutes spent in the different intensity intervals was proportionally adjusted to 14 h with the following equation: adjusted minutes=(observed in interval)×(14×60/total minutes) [17].

Values for all blood parameters at both baseline and follow-up are missing in one participant from the intervention group due to needle phobia. Insulin and HOMA values were regarded as missing for one

participant in the control group due to type 1-diabetes. Follow up cholesterol for one person and baseline HOMA for another participant from the intervention group were not obtained due to irregularity at the laboratory. Systolic blood pressure is missing in one participant due to resistance.

One participant from the intervention group performed maximal tests, but was not measured with the metabolic cart. $\dot{V}O_2$peak was in this case estimated from the regression equation between power output (MPO) and $\dot{V}O_2$peak of the study sample. Change in $\dot{V}O_2$peak was considered missing in 3 participants since test criteria was not met at follow-up.

Statistics

Crude baseline measurements were compared between the bicycling and the control group participants using unpaired mean comparison tests (t-tests), whereas paired t-tests were used in within group comparisons from baseline to follow-up.

Post intervention values were analysed across the two groups using univariate analysis of co-variance (ANCOVA), as suggested by Twisk and Proper[18], with participants grouped as originally randomised regardless of the degree of intervention compliance and types of activity actually performed. In efficacy analyses participants are referred to as compliant in the intervention group if at least 80% of possible trips to school were by bike on contrast participants in the control group are considered compliant if less than 20% of possible trips were by bike. Change in outcome was compared between the bicycling and the control group with adjustment for baseline measure and gender. All covariates were selected a priori and thus in any case kept in the statistical modeling even if non-significant. Regression analyses were preceded by verification of fulfillment of parametric assumptions by qq-plots and Shapiro Wilks tests.

Z scores (observed value - baseline mean / baseline SD) were computed for each of the variables included in the composite Z score. A high Z score value (or an increase) is considered to have adverse health effects for all variables included in the composite score with exception of cardiorespiratory fitness where inverse values were calculated. Composite Z scores, based on baseline and follow-up measurements, were constructed as a mean of the available standardised selected risk factors (fasting triglyceride, insulin sensitivity (HOMA), sum of four skinfolds, systolic blood pressure, total cholesterol/HDL ratio and inverse aerobic fitness). Composite baseline and follow-up Z scores were then standardised according to the baseline mean and SD allowing for interpretation of change in composite Z score. Subsequently the change in standardised composite Z score was calculated as (standardised mean of Z scores at follow up - standardised mean of Z scores at baseline).

Assuming a mean change of 10% in $\dot{V}O_2$peak and a SD of change of 0.3 L $O_2 \cdot min^{-1}$ the study needed 21 participants in both groups to be powered at 80%. All analyses were conducted using STATA IC version

11.0 (STATA Corp, College station) with alpha=0.05.

RESULTS

Background characteristics

Of the 54 participants randomized, 11 declined to participate (two due to moving, one due to personal reasons, one due to parental job situation, two due to test methods and five not accepting randomisation) leaving 43 participants (79.6%) in the study. No subjects experienced accidents or other harms. All 43 participants were measured at baseline and returned for follow-up (Fig. 1). Based on published age and gender BMI cut-off values [13] no participants were obese at baseline, whereas three and two participants were overweight in the bicycling and control group, respectively. There were no statistical differences between the control group and the bicycling group participants who completed the 8-week intervention period on any of the baseline features presented in Table 1, nor was there any baseline difference in the two primary dependent variables: $\dot{V}O_2$peak and mean of Z scores.

(insert table 1 here)

Adherence

All of the 43 allocated participants were available at follow-up assessments. Five participants in the intervention group and one in the control group were defined as non-compliant. The average compliance was 96.2% and 84.2 % in the control and the intervention group, respectively.

(insert table 2 here)

(insert table 3 here)

Change in $\dot{V}O_2$peak from baseline to follow-up

Crude comparisons of the effects within groups showed that $\dot{V}O_2$peak mean increased from 1.81 at baseline to 1.87 L $O_2 \cdot min^{-1}$ at follow-up (i.e. an increase by 3.3%) in the intervention group whereas the change in the control group was 1.69 vs. 1.73 L $O_2 \cdot min^{-1}$ (i.e. an increase by 2.3%). Based on adjusted ITT regression analyses children who started bicycling to school did not improve fitness (beta = 0.0336911, p= 0.458) compared to controls. Likewise based on adjusted estimates from efficacy analyses children who started bicycling to school did not improve fitness (beta = 0.0425191, p= 0.404) compared to controls. There was a significant association (p<0.001) between kilometers bicycled to school and fitness improvement (figure 2).

Change in composite Z score from baseline to follow-up

Crude comparisons of the effects within groups showed that the standardised composite Z score decreased from 0.01 at baseline to -0.26 at follow-up in the intervention group, whereas in the control group the corresponding change was from 0.01 to 0.28.

Based on adjusted ITT regression analyses children who started bicycling to school lowered their composite standardised Z score (beta = -0.58, p=0.012) compared to controls. Based on adjusted efficacy analyses children who started bicycling to school lowered their standardised composite Z score (beta = -0.63, p=0.015) compared to controls. There was no significant association (p=0.512) between kilometers bicycled to school and change in composite Z score.

Post hoc regression analyses of the 6 Z transformed delta variables included in the composite score showed that every single variable, although statistically non-significant individually, contributed to the lower standardised composite score in the intervention group. Standardised delta coefficients were: Inverse fitness -0.085867 (p= 0.458), systolic blood pressure -0.1074832 (p=0.644), sum of 4 skinfolds -0.1522508 (p=0.118), triglyceride -0.2713089 (p=0.295), cholesterol ratio -0.0405599 (p=0.838), HOMA score -0.4582449 (p=0.049).

(insert table 4 here)

DISCUSSION

Statement of principal findings

The main finding in this study was that bicycling to school for a period of 8 weeks significantly lowered clustering of cardiometabolic risk factors by 0.58 SD. The effect size estimate was different between the control and the intervention group in ITT analyses, though not all participants were fully compliant during the study period. The level of statistical significance of the clustered risk score was statistically significant even when multiple testing (due to two outcomes) was taken into account with the conservative Bonferroni method [19].

Post hoc regression analyses of the 6 Z transformed delta variables included in the composite score showed that every included variable contributed to the improved clustered score. This is noteworthy since it indicates a consistent positive effect of cycling to school on a range of various health parameters.

The composite risk factor score was used in order to compensate for the day-to-day fluctuation in the single risk factors and included the same variables as in previous studies by our group[6]. A continuous score as a proxy of the metabolic syndrome was chosen in favor of a dichotomous outcome in order to enhance statistical sensitivity, and because it is considered to describe the metabolic health condition better [20]. Further, none of the children had MS according to the definition by the International Diabetes Federation [21].

Bicycling to school did not have the expected effect on cardiorespiratory fitness per se since no significant difference between cyclists and controls was observed. This was contra intuitive since 4.6-5.9 % higher aerobic power has been reported in cross sectional studies comparing adolescents bicycling to school to walkers and passive transporters [22]. Furthermore a 9 % improvement has been found in children who started bicycling to school in prospective studies by Cooper et al. [23]. The discrepancy might be due to a relatively short intervention period in the present study and perhaps also because of too short distance to school. The latter being indirectly indicated from the significant association between kilometers bicycled to school and cardiorespiratory fitness improvement. In this connection it is noteworthy that a 7.3% improvement in $\dot{V}O_2$max [24] has been observed in a randomised study of adults who started bicycling to work and that the commuting trip on average was about 5km, which is twice the distance of the children included in this study. Finally, we cannot rule out that the non-significant difference in changes of $\dot{V}O_2$peak between groups could be a consequence of lack of statistical power (i.e. type II-error) since a change in $\dot{V}O_2$peak by 10% would require 21 perfectly compliant participants in both groups to be powered at 80%.

The preliminary power calculations in the present study were based on expected change in V̇O2peak since no previous data on the potential effect of bicycling to school on cardiometabolic health were available.

Compliance

Participants did not exhibit a behavior that was fully in accordance to the group they were randomised into. During the intervention period five participants in the intervention group bicycled less than 80% of all possible trips to school, and one participant in the control group cycled more than 20% of all trips to school by bike. A relative cutoff value of 80% was set allowing room for participants to be included in the efficacy analyses though subject to sickness, bike theft and the like. This arbitrary relative cutoff was preferred to an absolute due to slightly varying study duration and coincides well with the frequency of bicycling observed in the underlying population. In the ITT analyses we maintained the strengths of the RCT-design and accounted for potential known and unknown confounding through inclusion of all allocated participants in the analyses knowing that the true health potential of bicycling to school is likely to be larger than our estimates. In supplementary efficacy analyses we accounted for the non-compliance and found slightly larger effect estimates for both composite Z score and $\dot{V}O_2$peak. We included "non-bicycling" participants based on self-reported mode of transportation to school. From the transport diaries it was possible to assess whether participants in the period preceding the study had in fact been non-bicycling and meeting inclusion criteria (as self-claimed). Four participants in the control group and one in the intervention unfortunately bicycled more than 20% of possible trips to school in the two weeks pre-baseline period. Participants were kept in both ITT and efficacy-analyses though their appearance possibly diluted the true effect of commuter bicycling to school. It is evident that acceptance and understanding of randomisation of transport to school was a challenge. Transport to school is an integrated part of the daily logistics of the entire family making compliance highly susceptible to various factors such as parental job situation, parental marital status and sudden extra vacation.

Strengths and weaknesses of the study

The major strength of this study was the randomised design which through elimination of bias allowed for investigation of a causal relationship between bicycling to school and health outcomes. Compliance was supported by inclusion of participants from numerous classes consequently diminishing the risk of contamination between groups. Direct measurements of $\dot{V}O_2$peak and all baseline and follow-up measurements being carried out by the same experienced, and blinded test personnel are likewise study strengths. Furthermore detailed information on the degree of exposure before (allowing assessment of fulfillment of inclusion criteria) and during (e.g. mode of transportation to school, amount of bicycling to

school, amount of bicycling beyond commuter bicycling, commuter bicycling intensity, general physical activity level) the study is advantageous. A study weakness was a relatively small study sample not behaving perfectly in accordance to the group randomised into, and consequently possible compromised the detection of an effect of bicycling to school on cardiorespiratory fitness. It is likely that the amount of total bicycling assessed from SMS-reported odometer status were underestimated because some odometers reset due to malfunction. Possible this underestimation is biased as the intervention group generally bicycled more trips and thus was more susceptible to theft and malfunction. This, however, is not crucial in the present study focusing on commuter bicycling being assessed from the transport diaries. Field measurements of commuter bicycling intensity and distance are based solely on one single trip to school and should therefore be taken with caution. Finally we cannot rule out that the field measurements have been biased due to the Hawthorne effect (i.e. children may have changed their behavior as they knew they were being studied). A possible Hawthorne effect should, however, beside from the field measurements be equal in the two study arms.

Recruitment

It was feasible to carry out the present study because it is relatively safe and feasible to bicycle to school in Denmark. The recruitment of participants, however, turned out to be rather difficult partly due to the fact that approximately 60% of children in the region already bicycled to school[9] and thus not includable. From the remaining 40% some lived too far away from the school to bicycle, while others lived so close that they preferred to continue walking. Others were unable or unwilling to have their daily transport dictated by a randomisation. The recruitment difficulties forced us to include participants living closer to the school than our pre-determined inclusion criteria of 2km. The inference of inclusion of children with short commuter distance is that the observed effect of the present study had greater external validity.

Exposure in the intervention group

In the field test children bicycled to school at a self-chosen average intensity of 73.6% of maximal heart rate, which is similar to Finnish adults who commute at a relative intensity of approximately 73.5-78.6%[25]. The relative intensities for adult commuting were converted from %VO_2peak to %maximal heart rate by means of the regression equation reported by Swain[26]. Interestingly, web-assessed distance to school was 2.27 km (SD = 1.55) in the intervention group, whereas GPS-assessed distance in the same group was 2.13 km (SD = 1.51) ($p = 0.08$). Thus, though needed to be investigated further, self-reported commuter distance in children seem to be valid with (SD delta variable = 0.34).

Despite short school travelling distance, short study duration and non-perfect compliance among the participants included in our ITT-analyses, a significantly decrease in sum of Z score was found in the intervention group compared to the control group. Accelerometer measurements conducted midway in the study indicated that this decrease was not mediated through an increased level of general physical activity since neither mean count nor adjusted minutes at various intensity levels (the latter not included in the results section) were statistically significant different between groups. Our rationale for including children walking to school was based on previous studies reporting no significant differences in various measures of physical fitness between participants walking to school and those using passive transport[8,22].

Main message

Though comparison of effect sizes should be done with caution[27] a lowering of the composite Z score by 0.58 is substantial. In comparison an increase in continuous metabolic syndrome score of 1 has been associated with an odds ratio of developing type-2 diabetes mellitus of 3.4 and 5.1 in men and women, respectively, and odds ratio for cardiovascular disease of 1.7[28]. Consequently a lowering of metabolic syndrome score by 0.58 could theoretically lower the odds of developing diabetes about 45% and CVD around 25%.

In conclusion bicycling to school had positive effect on clustering of cardiometabolic risk factors in children and should thus be considered as effective prevention of diabetes and cardiovascular disease.

Figure legends:

Figure 1. Participants flow diagram

Figure 2. Dose-response between bicycling to school and fitness improvement in the intervention group.

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Contributors

LØ designed the study, performed the randomisation, analysed and interpreted data, drafted the manuscript, and is guarantor. JT collected, analysed and interpreted the data and revised the manuscipt. LAB revised the manuscript. LBA interpreted data and revised the manuscript. All authors approved the submitted version of the manuscript.

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Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work.

Ethical approval

The study was approved by the regional Ethics Committee. Written informed consent was obtained from the child's parent or legal guardian.

Data sharing statement

Data will not be publically accessible. However, interested individuals may contact the corresponding author.

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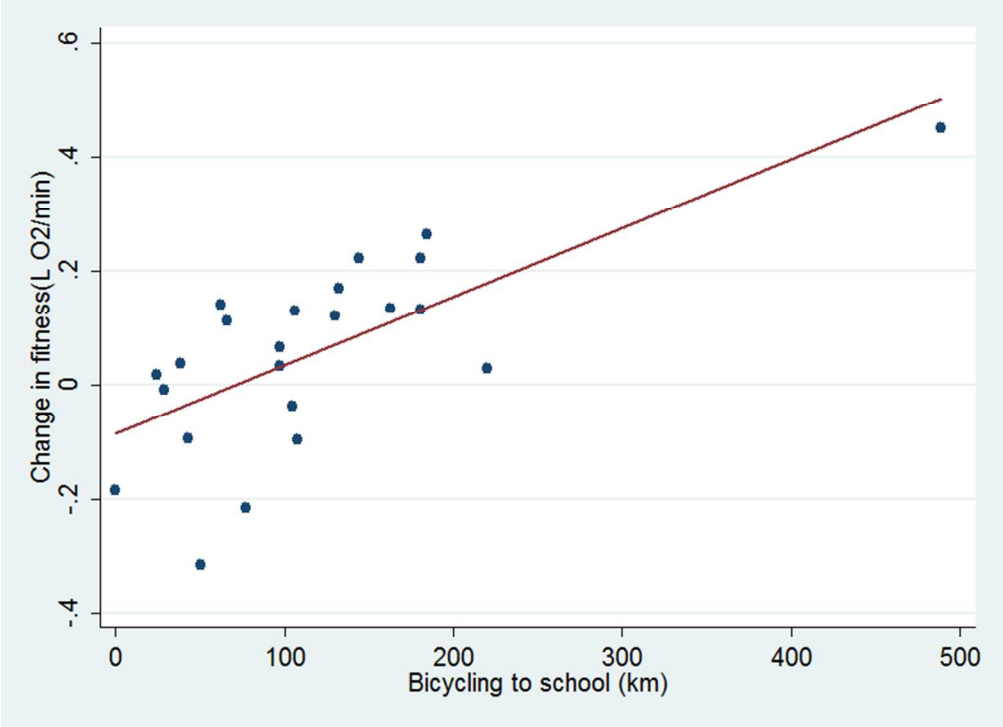

Figure 2. Dose-response between bicycling to school and fitness improvement in the intervention group
281x204mm (72 x 72 DPI)

Table 1.Baseline characteristics in the intervention and control group by gender

Bicycling group	Boys (n=14)	Girls (n=9)	Total (n=23)
Age (years)	12.2 (0.9)	11.8 (0.7)	12.0 (0.8)
Height (cm)	152.4 (8.4)	152.4 (4.7)	152.4 (7.1)
Weight (kg)	44.2 (8.2)	44.0 (7.2)	44.1 (7.6)
BMI ($kg \cdot m^{-2}$)	18.9 (2.1)	18.9 (2.4)	18.9 (2.2)
Distance to school (km)	2.43 (1.75)	1.95 (1.10)	2.24 (1.52)
Activity level (counts/min)	661 (215)	477 (124)	589 (204)
Control group	Boys (n=12)	Girls (n=8)	Total (n=20)
Age (years)	11.9 (0.8)	11.6 (0.7)	11.8 (0.8)
Height (cm)	150.3 (7.0)	150.2 (6.6)	150.3 (6.7)
Weight (kg)	41.0 (7.5)	42.1 (7.4)	41.5 (7.3)
BMI ($kg \cdot m^{-2}$)	18.1 (2.4)	18.6 (2.5)	18.3 (2.4)
Distance to school (km)	1.94 (1.52)	4.4 (4.5)	2.9 (3.2)
Activity level (counts/min)	531 (124)	547 (122)	537 (121)

Data presented as mean and (SD) values

Table 2. Number of bicycling trips to and from school accomplished by participants in the intervention and the control group

No. of trips	Bicycle group participants (n)	Control group participants (n)
0	1	17
1-9	1	1
10-19	0	1
20-29	0	0
30-39	1	1
40-49	1	0
50-59	10	0
60-69	8	0
70-79	1	0
Total	23	20

Table 3. Absolute and relative bicycling intensity, speed and distance to school by gender in the entire intervention group (i.e. both compliant and non-compliant participants included)

	Average intensity (bp/min)	Peak intensity (bp/min)	Average intensity (% of max HR)	Relative peak intensity (% of max HR)	Average speed (km/t)	School bicycling (km)
Boys	138.5 (15.8)	164.4 (17.9)	72.0 (7.4)	85.5 (8.7)	13.1(3.4)	124.4 (119.5)
Girls	146.6 (17.3)	171.9 (17.0)	75.2 (7.7)	88.2 (7.1)	13.9 (4.2)	109.7 (63.4)

Data presented as mean and (SD) values

Table 4. Measurements of risk factors at baseline and follow-up in the bicycling and the control group

	Bicycling group					Control group				
	Baseline		Follow-up			Baseline		Follow-up		
	Mean	SD	Mean	SD	p value	Mean	SD	Mean	SD	p value
$\dot{V}O_2$peak (L $O_2 \cdot min^{-1}$)	1.81	0.42	1.87	0.38	0.1180	1.69	0.36	1.73	0.34	0.1413
Systolic (mm Hg)	107.1	9.6	107.3	8.3	0.9188	105.0	8.7	107.6	8.7	0.2412
Diastolic (mm Hg)	60.8	7.0	61.2	4.1	0.7909	58.0	4.7	62.2	6.1	0.0036
BMI(kg $\cdot m^{-2}$)	18.9	2.2	19.1	2.3	0.0965	18.3	2.4	18.4	2.3	0.0427
Sum of four skinfolds (mm)	41.0	16.4	39.1	14.8	0.2057	36.3	15.5	37.6	15.6	0.2614
Total cholesterol(mmol/L)	3.93	0.63	4.16	0.60	0.0131	3.96	0.71	4.26	0.76	0.0023
HDL cholesterol(mmol/L)	1.48	0.34	1.53	0.37	0.1713	1.36	0.24	1.42	0.23	0.1256
LDL cholesterol(mmol/L)	2.20	0.61	2.27	0.57	0.2697	2.36	0.66	2.53	0.71	0.0379
Triglycerides(mmol/L)	0.64	0.17	0.67	0.22	0.3293	0.70	0.25	0.77	0.23	0.1181
Glucose(mmol/L)	5.35	0.26	5.48	0.44	0.1587	5.54	1.24	5.77	1.40	0.0036
Insulin (pmol/L)	42.10	28.48	33.68	17.88	0.0450	32.74	10.16	39.21	20.48	0.0971
HOMA score	1.70	1.25	1.41	0.85	0.1197	1.28	0.43	1.60	0.87	0.0622
Total cholesterol/HDL-ratio	2.75	0.53	2.82	0.58	0.2610	2.96	0.54	3.04	0.61	0.3363
Activity level (counts/min)	586	208	596	205	0.6668	537	120	577	184	0.3304
Standardised composite Z score	0.01	1.02	-0.26	1.32	0.0841	0.01	1.01	0.28	1.12	0.1081

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	13
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	15
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5+12

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	5+figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	5
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	12
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	10
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

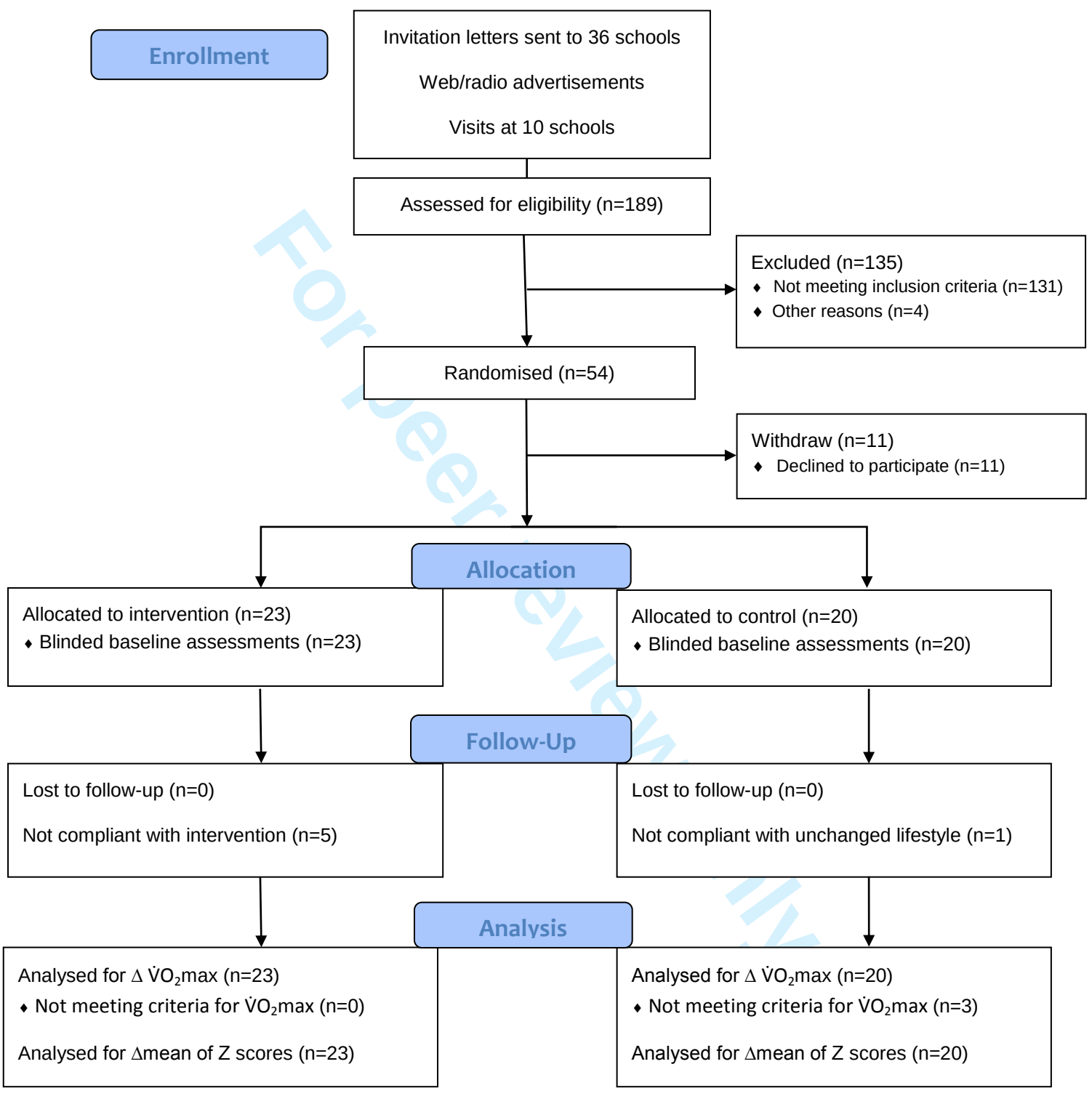

Figure 1

Bicycling to school improves the cardiometabolic risk factor profile: a randomised controlled trial

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Primary Subject Heading:	Public health
Secondary Subject Heading:	Epidemiology, Sports and exercise medicine, Cardiovascular medicine
Keywords:	cardiometabolic, risk factors, bicycling, children, commuting

TITLE PAGE

Bicycling to school improves the cardiometabolic risk factor profile: a randomised controlled trial

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Keywords: cardiometabolic, risk factors, bicycling, children, commuting

Word count: Abstract: 199, Main text: 4.343

ABSTRACT

Objectives:

To investigate whether bicycling to school improve cardiometabolic risk factor profile and cardiorespiratory fitness among children.

Design:

Prospective, blinded, randomised controlled trial.

Setting:

Single centre study in Odense, Denmark.

Participants:

43 children previously not bicycling to school were randomly allocated to control group (n=20) (i.e. no change in lifestyle) or intervention group (i.e. bicycling to school) (n=23).

Primary and secondary outcome measures:

Change in cardiometabolic risk factor score and change in cardiorespiratory fitness.

Results:

All participants measured at baseline returned at follow-up. Based upon intention to treat analyses, clustering of cardiometabolic risk factors was lowered by 0.58 SD (95% CI -1.03 to -0.14, p= 0.012) in the bicycling group compared to the control group. Cardiorespiratory fitness (L $O_2 \cdot min^{-1}$) per se did not increase significantly more in the intervention than in the control group (beta = 0.0337, 95% CI -0.06 to 0.12, p=0.458).

Conclusions:

Bicycling to school counteracted a clustering of cardiometabolic risk factors and should thus be recognized as potential prevention of type 2 diabetes mellitus and cardiovascular disease. The intervention did, however, not elicit a larger increase in cardiorespiratory fitness in the intervention group as compared to the control group.

Trial registration:

Registered at www.clinicaltrials.gov (NCT01236222)

ARTICLE SUMMARY

Article focus

- Intervention studies investigating whether bicycling to school in fact cause improved cardiometabolic health have not yet been carried out.
- The aim of this study was to investigate whether bicycling to school cause an increase in cardiorespiratory fitness and induce improvement of clustering of cardiometabolic risk factors

Key messages

- This study is the first study to document that bicycling to school had positive effect on clustering of cardiometabolic risk factors in children.

Strengths and Limitations

- The major strength of this study was the randomised design which through elimination of bias allowed for investigation of a causal relationship between bicycling to school and health outcomes.
- Relatively weak statistical power and impossibility of blindness of the treatment.

INTRODUCTION

The metabolic syndrome (MS) is the concurrence of multiple risk factors associated with cardiovascular disease and type 2 diabetes mellitus. Prevalence is between 20-30% of the adult population in most countries[1], and a rising incidence in children and adolescents[2], indicates that the condition represents a major threat to global public health. Exposure of the MS confers a doubled risk of incident cardiovascular event and death[3] and up to 5-times higher risk of developing type 2 diabetes mellitus in adults[4]. Furthermore, since clustering of cardiometabolic risk factors track from childhood to adulthood[5] early prevention of the MS is important in order to prevent occurrence of disease later in life.

In children and adolescents physical activity has the potential to prevent a clustering of risk factors[6], underpinned by the notion that an increase of daily moderate physical activity by 10-20% is associated with 33% lower risk of having the MS[7].

Active commuting such as walking and bicycling to school might be important contributors of preventive physical activity. Observational studies indicate that bicycling to school is beneficial to health since higher cardiorespiratory fitness[8] and lower BMI[9] have been observed in children bicycling to school compared to non-cyclists. If bicycling to school is generally acknowledged as a time-efficient and feasible form of daily physical activity it could potentially target the entire population. From a public health perspective this would be very appealing considering that the greatest health benefits are achieved in the least active individuals [10].

There seems to be wide-ranging scepticism about the opportunity to bicycle to school in industrialized societies. The level of concern, however seems often to be negatively associated with the prevalence of bicycling to school, which in Denmark for instance is about 60% among adolescents [8,9]whereas in the UK approximately 2% bicycle to school [11].

Intervention studies investigating whether bicycling to school in fact cause improved cardiometabolic health have not yet been carried out. One reason for this could be that very few countries have an infra-structure allowing safe commuting by bike to school. Another reason could be that interventions often include change in the built environment in order to provide safe routes and this is difficult to control in a rigid study design.

The evidence linking bicycle commuting with cardiometabolic health in children is still limited and experimental studies investigating causality have been requested [12]. Hence, the aim of this study is to investigate, in a randomised trial, whether bicycling to school cause an increase in cardiorespiratory fitness and induce improvement of clustering of cardiometabolic risk factors.

METHODS

Study participants

This study took place in Denmark in the municipality of Odense during spring 2011. Participants were recruited through invitation letters sent to 36 elementary public schools in the municipality of Odense. Investigators then visited 10 of these schools (approximately 92 classes) and presented the project. Additionally, the project was presented online and in a radio spot. Participants were included if they at the time of registration stated that they had not bicycled regularly to and from school for at least 3 months (i.e. at least from January onwards) prior to the intervention, and if willing to be randomised to one of the two study groups (i.e. control group or bicycling group). Reasons for exclusion were not having a bike, and/or affirming less than one km between home and school. One hundred and eighty-nine children volunteered to participate in the study. One hundred and thirty-one of these, however, claimed that they bicycled regularly to school, and four withdrew from the study due to a changed family situation. Fifty-four eligible children were subsequently randomised to either control or intervention. After randomisation six and five participants from control and intervention group, respectively, withdrew since they did not accept being randomized, leaving 43 participants in the study (Figure 1). Written informed consent was obtained from the child's parent or legal guardian after they were given a detailed written explanation of the aims of the study, possible hazards, discomfort, and inconvenience, and the option to withdraw at any time. The study was approved by the regional Ethics Committee (S-20100009) and registered at clinicaltrials.gov (NCT01236222) with the purpose of investigating the effect of bicycling to school on the metabolic syndrome and aerobic fitness.

Study design and intervention

Participants were randomly assigned to a control group (i.e. no change in lifestyle) or to an experimental group (i.e. commuter bicycling). A permuted random block size (2, 4 and 6) randomisation (www.randomization.com) was used in order to reduce the chances of the assignment schedule being seen by those responsible for recruitment of participants[13]. Twenty participants were allocated to the control group and 23 participants to the bicycling group. All measurements were repeated at the conclusion of the 8-week intervention program. There were no restrictions beside the mode of transportation to school. The study period comprised one week of national holiday and additionally two bank holidays where no children attended school. Matching baseline test date with follow-up test (in order to achieve similar intervention duration between subjects) was strived for, but logistically not possible for all participants. This has implications on the amount of exposure the intervention group was able to accumulate. Participants could maximally accumulate between 56 and 74 trips to and from school during

the study period. All measurements at baseline and follow-up were conducted by personal blinded to group allocation. Children were picked up and returned to their home addresses when scheduled for baseline and follow-up tests.

Measurements

Height was measured by a stadiometer (SECA, Hamburg, Germany), children wearing socks or bare feet and weight was assessed by a 0.1 kg precision scale (Soehnle Professional, Murrhardt, Germany) wearing only shorts and t-shirts. Skinfold thickness was measured on the left side with a Harpenden caliper (John Bull, British Indicators Ltd., West Sussex, England) at the biceps, triceps, subcapsular, and suprailiac sites. Overweight/obesity status was defined according to age- and gender specific published cut-points for BMI[14].

Systolic and diastolic blood pressure was measured by a sphygmomanometer (DINAMAP ProCare 100, General electric, USA). At least five measurements were made on the left arm with two min interval and the mean of the final three measurements were used as systolic and diastolic pressure.

All Children were instructed to fast overnight (>8 hours), and only allowed to drink water until the blood sample was drawn. Blood was drawn from the right fossa cubitus by a biomedical laboratory scientist. If a participant had not been fasting or if he/she had experienced any illness during the last week, a new test day was scheduled for that child. Samples were analysed for: Glucose, insulin, cholesterol, triglyceride. Insulin resistance was estimated according to the homoeostasis model assessment (HOMA) as the product of fasting glucose (mmol/L) and insulin (µU/mL) divided by the constant 22.5[15]. Breakfast was served on site subsequent of blood sampling. All analysis of the blood specimens were conducted at Department of Clinical Biochemistry and Pharmacology, Odense Universityhospital

Aerobic fitness ($\dot{V}O_2$peak) was determined in a progressive bicycle test with on an electronically braked ergometer (Monark 839 Ergomedic, Varberg, Sweden). Pulmonary gas exchange was measured with a metabolic cart (Amis2001, Innovision, Denmark) with 15 seconds epoch, and the highest value within 30 seconds was regarded as a maximal if respiratory exchange ratio (RER) >= 0.99 or maximal heart rate (HR) was >=185 beats/min, and the test leader judged the participant to show signs of intense effort (e.g. facial flushing or difficulties in keeping up the pedal frequency)[16]. HR was measured with a HRM (Polar RS800CX, Kempele, Finland) with epoch set to 1 sec. Children were thoroughly explained the purpose of the test and that it would require a maximal effort. After a warm-up period of 5 minutes at 40W, work-load was increased 40W every 2 minutes until volitional exertion. Verbal encouragement was given throughout the test and recommended pedaling cadence was 60-80 rpm.

Children completed a general questionnaire regarding transportation to school, sports-habits, and general quality of life. At follow up all children marked their route to school using a web-based map tool (www.loebererute.dk).

All children had their bike equipped with an odometer on average two weeks before baseline measurement. The odometers were individually calibrated in accordance to wheel circumference. Odometer levels were self-reported, by participants in both groups, every Sunday until tested at follow-up by means of a commercial SMS system (SMS-Track ApS). Participants who did not answer Sunday were contacted Monday. Malfunctioning odometers were replaced within a few days.

Both groups were instructed to report daily mode of school transportation on a custom made transport diary. Total mileage of school related bicycling during the study was calculated from the distance to school (web route assessment) times the number of trips to/from school (transport diary).

Field measurent of intensity of bicycling to school was carried out midway (five weeks after baseline). Participants in the intervention group received a GPS (Qstarz BT-Q1300S, Qstarz International Inc., Taiwan) and a heart rate monitor (HRM) (PolarTeam^2, Polar, Kempele, Finland) and were instructed to wear the devices during one day. Both GPS and HRM were set at an epoch of 5 second. All GPS data were transferred to commercial software (Travel recorder v. 5.0) and corrected for drift using manufacturer software. The GPS was the reference for all HRM data. Mean HR of school transportation, if verified by the diary, was calculated as the mean of the measurements from the first data point when the child exceeded a speed of 5 km/h when leaving home, to the last data point above 5 km/h when the child arrived at school. Both compliant and non-compliant participants were included in the determination of commuter bicycling intensity since the primary outcome is based on intention to treat (ITT) analyses.

Physical activity beyond bicycling was assessed with an accelerometer (Actigraph GT3X, Manufacturing Technology Inc, Fort Walton Beach, FL, USA). Physical activity was monitored for 7 consecutive days from Monday to Sunday at baseline and midway (five weeks after baseline). Instruments were attached at the hip, and counts were sampled every two seconds. Data reduction was performed with customised software (Propero, Odense, Denmark). Only data from the vertical axis was included in the data analyses. Criteria for a successful recording were a minimum of 3 days of 10 h recording per day. Time periods of at least 30 consecutive minutes of zero counts were deemed to represent periods when the monitor was not worn and thus disregarded. Cut points for intensity levels were based on the Freedson/Trost equation[17]. Since cutpoints for physical activity intensity are specifically designed for 1 min epoch these were divided by 30. The average time the accelerometer was worn was 13.5 and 13.6 h per day at baseline and follow up, respectively, and the number of minutes spent in the different intensity

intervals was proportionally adjusted to 14 h with the following equation: adjusted minutes=(observed in interval)×(14×60/total minutes) [18].

Values for all blood parameters at both baseline and follow-up are missing in one participant from the intervention group due to needle phobia. Insulin and HOMA values were regarded as missing for one participant in the control group due to type 1-diabetes. Follow up cholesterol for one person and baseline HOMA for another participant from the intervention group were not obtained due to irregularity at the laboratory. Systolic blood pressure is missing in one participant due to resistance.

One participant from the intervention group performed maximal tests, but was not measured with the metabolic cart. $\dot{V}O_2$peak was in this case estimated from the regression equation between power output (MPO) and $\dot{V}O_2$peak of the study sample. Change in $\dot{V}O_2$peak was considered missing in 3 participants since test criteria was not met at follow-up.

Statistics

Crude baseline measurements were compared between the bicycling and the control group participants using unpaired mean comparison tests (t-tests), whereas paired t-tests were used in within group comparisons from baseline to follow-up.

Post intervention values were analysed across the two groups using analysis of co-variance (ANCOVA), as suggested by Twisk and Proper[19], with participants grouped as originally randomised regardless of the degree of intervention compliance and types of activity actually performed. In efficacy analyses participants are referred to as compliant in the intervention group if at least 80% of possible trips to school were by bike on contrast participants in the control group are considered compliant if less than 20% of possible trips were by bike. Change in outcome was compared between the bicycling and the control group with adjustment for baseline measure and gender. All covariates were selected a priori and thus in any case kept in the statistical modeling even if non-significant. Regression analyses were preceded by verification of fulfillment of parametric assumptions by qq-plots and Shapiro Wilks tests.

Z scores (observed value - baseline mean / baseline SD) were computed for each of the variables included in the composite Z score. A high Z score value (or an increase) is considered to have adverse health effects for all variables included in the composite score with exception of cardiorespiratory fitness where inverse values were calculated. Composite Z scores, based on baseline and follow-up measurements, were constructed as a mean of the available standardised selected risk factors (fasting triglyceride, insulin sensitivity (HOMA), sum of four skinfolds, systolic blood pressure, total cholesterol/HDL ratio and inverse aerobic fitness). Composite baseline and follow-up Z scores were then standardised according to the baseline mean and SD allowing for interpretation of change in composite Z score. Subsequently the change

in standardised composite Z score was calculated as (standardised mean of Z scores at follow up - standardised mean of Z scores at baseline).

Assuming a mean change of 10% in $\dot{V}O_2$peak and a SD of change of 0.3 L $O_2 \cdot min^{-1}$ the study needed 21 participants in both groups to be powered at 80%. All analyses were conducted using STATA IC version 11.0 (STATA Corp, College station) with alpha=0.05.

RESULTS

Background characteristics

Of the 54 participants randomized, 11 declined to participate (two due to moving, one due to personal reasons, one due to parental job situation, two due to test methods and five not accepting randomisation) leaving 43 participants (79.6%) in the study. No subjects experienced accidents or other harms. All 43 participants were measured at baseline and returned for follow-up (Fig. 1). Based on published age and gender BMI cut-off values [14] no participants were obese at baseline, whereas three and two participants were overweight in the bicycling and control group, respectively. There were no statistical differences between the control group and the bicycling group participants who completed the 8-week intervention period on any of the baseline features presented in Table 1, nor was there any baseline difference in the two primary dependent variables: $\dot{V}O_2$peak and mean of Z scores.

Table 1. Baseline characteristics in the intervention and control group by gender

Bicycling group	Boys (n=14)	Girls (n=9)	Total (n=23)
Age (years)	12.2 (0.9)	11.8 (0.7)	12.0 (0.8)
Height (cm)	152.4 (8.4)	152.4 (4.7)	152.4 (7.1)
Weight (kg)	44.2 (8.2)	44.0 (7.2)	44.1 (7.6)
BMI ($kg \cdot m^{-2}$)	18.9 (2.1)	18.9 (2.4)	18.9 (2.2)
Distance to school (km)	2.43 (1.75)	1.95 (1.10)	2.24 (1.52)
Activity level (counts/min)	661 (215)	477 (124)	589 (204)
Control group	Boys (n=12)	Girls (n=8)	Total (n=20)
Age (years)	11.9 (0.8)	11.6 (0.7)	11.8 (0.8)
Height (cm)	150.3 (7.0)	150.2 (6.6)	150.3 (6.7)
Weight (kg)	41.0 (7.5)	42.1 (7.4)	41.5 (7.3)
BMI ($kg \cdot m^{-2}$)	18.1 (2.4)	18.6 (2.5)	18.3 (2.4)
Distance to school (km)	1.94 (1.52)	4.4 (4.5)	2.9 (3.2)
Activity level (counts/min)	531 (124)	547 (122)	537 (121)

Data presented as mean and (SD) values

Adherence

All of the 43 allocated participants were available at follow-up assessments. Five participants in the intervention group and one in the control group were defined as non-compliant. The average compliance was 96.2% and 84.2 % in the control and the intervention group, respectively.

Table 2. Number of bicycling trips to and from school accomplished by participants in the intervention and the control group

No. of trips	Bicycle group participants (n)	Control group participants (n)
0	1	17
1-9	1	1
10-19	0	1
20-29	0	0
30-39	1	1
40-49	1	0
50-59	10	0
60-69	8	0
70-79	1	0
Total	23	20

Table 3. Absolute and relative bicycling intensity, speed and distance to school by gender in the entire intervention group (i.e. both compliant and non-compliant participants included)

	Average intensity (bp/min)	Peak intensity (bp/min)	Average intensity (% of max HR)	Relative peak intensity (% of max HR)	Average speed (km/t)	School bicycling (km)
Boys	138.5 (15.8)	164.4 (17.9)	72.0 (7.4)	85.5 (8.7)	13.1(3.4)	124.4 (119.5)
Girls	146.6 (17.3)	171.9 (17.0)	75.2 (7.7)	88.2 (7.1)	13.9 (4.2)	109.7 (63.4)

Data presented as mean and (SD) values

Change in $\dot{V}O_2$peak from baseline to follow-up

Crude comparisons of the effects within groups showed that $\dot{V}O_2$peak mean increased from 1.81 at baseline to 1.87 L $O_2 \cdot min^{-1}$ at follow-up (i.e. an increase by 3.3%) in the intervention group whereas the change in the control group was 1.69 vs. 1.73 L $O_2 \cdot min^{-1}$ (i.e. an increase by 2.3%). Based on adjusted ITT regression analyses children who started bicycling to school did not improve fitness (beta = 0.0336911, p= 0.458) compared to controls. Likewise based on adjusted estimates from efficacy analyses children who started bicycling to school did not improve fitness (beta = 0.0425191, p= 0.404) compared to controls. There was a significant association (p<0.001) between kilometers bicycled to school and fitness improvement (figure 2).

Change in composite Z score from baseline to follow-up

Crude comparisons of the effects within groups showed that the standardised composite Z score decreased from 0.01 at baseline to -0.26 at follow-up in the intervention group, whereas in the control group the corresponding change was from 0.01 to 0.28.

Based on adjusted ITT regression analyses children who started bicycling to school lowered their composite standardised Z score (beta = -0.58, p=0.012) compared to controls. Based on adjusted efficacy analyses children who started bicycling to school lowered their standardised composite Z score (beta = -0.63, p=0.015) compared to controls. There was no significant association (p=0.512) between kilometers bicycled to school and change in composite Z score.

Post hoc regression analyses of the 6 Z transformed delta variables included in the composite score showed that every single variable, although statistically non-significant individually, contributed to the lower standardised composite score in the intervention group. Standardised delta coefficients were: Inverse fitness -0.085867 (p= 0.458), systolic blood pressure -0.1074832 (p=0.644), sum of 4 skinfolds -

0.1522508 (p=0.118), triglyceride -0.2713089 (p=0.295), cholesterol ratio -0.0405599 (p=0.838), HOMA score -0.4582449 (p=0.049).

DISCUSSION

Statement of principal findings

The main finding in this study was that bicycling to school for a period of 8 weeks significantly lowered clustering of cardiometabolic risk factors by 0.58 SD. The effect size estimate was different between the control and the intervention group in ITT analyses, though not all participants were fully compliant during the study period. The level of statistical significance of the clustered risk score was statistically significant even when multiple testing (due to two outcomes) was taken into account with the conservative Bonferroni method [20].

Standardized residuals were plotted against the predicted values and no systematic patterns were observed which confirmed variance-homogeneity. QQ plots and Shapiro wilks tests of the standardized residuals of the model expressed normality. The goodness of fit as indicated by r-squared values in the regression modeling of change in the standardised composite Z score were 0.16 and 0.19 for ITT and efficacy analyses respectively. Goodness of fit for the modeling of change in $\dot{V}O_2$peak was 0.17 and 0.18 for ITT and efficacy analyses respectively (for additional results from the regression analyses see supplement 2).

Post hoc regression analyses of the 6 Z transformed delta variables included in the composite score showed that every included variable contributed to the improved clustered score. This is noteworthy since it indicates a consistent positive effect of cycling to school on a range of various health parameters.

The composite risk factor score was used in order to compensate for the day-to-day fluctuation in the single risk factors and included the same variables as in previous studies by our group[6]. A continuous score as a proxy of the metabolic syndrome was chosen in favor of a dichotomous outcome in order to enhance statistical sensitivity, and because it is considered to describe the metabolic health condition better [21]. Further, none of the children had MS according to the definition by the International Diabetes Federation [22].

Bicycling to school did not have the expected effect on cardiorespiratory fitness per se since no significant difference between cyclists and controls was observed. This was contra intuitive since 4.6-5.9 % higher aerobic power has been reported in cross sectional studies comparing adolescents bicycling to school to walkers and passive transporters [23]. Furthermore a 9 % improvement has been found in children who started bicycling to school in prospective studies by Cooper et al. [24]. The discrepancy might be due to a relatively short intervention period in the present study and perhaps also because of too short distance to school. The latter being indirectly indicated from the significant association between kilometers bicycled to school and cardiorespiratory fitness improvement. In this connection it is noteworthy that a 7.3%

improvement in $\dot{V}O_2$max [25] has been observed in a randomised study of adults who started bicycling to work and that the commuting trip on average was about 5km, which is twice the distance of the children included in this study. Interestingly post-hoc linear regression showed that both the relative average and the relative maximal intensity during commuter bicycling was positively associated (see data supplement 1) with cardiorespiratory fitness improvements (p=0.005 and p=0.002 respectively).

Finally, we cannot rule out that the non-significant difference in changes of $\dot{V}O_2$peak between groups could be a consequence of lack of statistical power (i.e. type II-error) since a change in $\dot{V}O_2$peak by 10% would require 21 perfectly compliant participants in both groups to be powered at 80%. The preliminary power calculations in the present study were based on expected change in $\dot{V}$O2peak since no previous data on the potential effect of bicycling to school on cardiometabolic health were available.

Compliance

Participants did not exhibit a behavior that was fully in accordance to the group they were randomised into. During the intervention period five participants in the intervention group bicycled less than 80% of all possible trips to school, and one participant in the control group cycled more than 20% of all trips to school by bike. A relative cutoff value of 80% was set allowing room for participants to be included in the efficacy analyses though subject to sickness, bike theft and the like. This arbitrary relative cutoff was preferred to an absolute due to slightly varying study duration and coincides well with the frequency of bicycling observed in the underlying population. In the ITT analyses we maintained the strengths of the RCT-design and accounted for potential known and unknown confounding through inclusion of all allocated participants in the analyses knowing that the true health potential of bicycling to school is likely to be larger than our estimates. In supplementary efficacy analyses we accounted for the non-compliance and found slightly larger effect estimates for both composite Z score and $\dot{V}O_2$peak. We included “non-bicycling” participants based on self-reported mode of transportation to school. From the transport diaries it was possible to assess whether participants in the period preceding the study had in fact been non-bicycling and meeting inclusion criteria (as self-claimed). Four participants in the control group and one in the intervention unfortunately bicycled more than 20% of possible trips to school in the two weeks pre-baseline period. Participants were kept in both ITT and efficacy-analyses though their appearance possibly diluted the true effect of commuter bicycling to school. It is evident that acceptance and understanding of randomisation of transport to school was a challenge. Transport to school is an integrated part of the daily logistics of the entire family making compliance highly susceptible to various factors such as parental job situation, parental marital status and sudden extra vacation.

Strengths and weaknesses of the study

The major strength of this study was the randomised design which through elimination of bias allowed for investigation of a causal relationship between bicycling to school and health outcomes. Compliance was supported by inclusion of participants from numerous classes consequently diminishing the risk of contamination between groups. Direct measurements of $\dot{V}O_2$peak and all baseline and follow-up measurements being carried out by the same experienced, and blinded test personnel are likewise study strengths. Furthermore detailed information on the degree of exposure before (allowing assessment of fulfillment of inclusion criteria) and during (e.g. mode of transportation to school, amount of bicycling to school, amount of bicycling beyond commuter bicycling, commuter bicycling intensity, general physical activity level) the study is advantageous. A study weakness was a relatively small study sample not behaving perfectly in accordance to the group randomised into, and consequently possible compromised the detection of an effect of bicycling to school on cardiorespiratory fitness. It is likely that the amount of total bicycling assessed from SMS-reported odometer status were underestimated because some odometers reset due to malfunction. Possible this underestimation is biased as the intervention group generally bicycled more trips and thus was more susceptible to theft and malfunction. This, however, is not crucial in the present study focusing on commuter bicycling being assessed from the transport diaries. Field measurements of commuter bicycling intensity and distance are based solely on one single trip to school and should therefore be taken with caution. Finally we cannot rule out that the field measurements have been biased due to the Hawthorne effect (i.e. children may have changed their behavior as they knew they were being studied). A possible Hawthorne effect should, however, beside from the field measurements be equal in the two study arms.

Recruitment

It was feasible to carry out the present study because it is relatively safe and feasible to bicycle to school in Denmark. The recruitment of participants, however, turned out to be rather difficult partly due to the fact that approximately 60% of children in the region already bicycled to school[9] and thus not includable. From the remaining 40% some lived too far away from the school to bicycle, while others lived so close that they preferred to continue walking. Others were unable or unwilling to have their daily transport dictated by a randomisation. The recruitment difficulties forced us to include participants living closer to the school than our pre-determined inclusion criteria of 2km. The inference of inclusion of children with short commuter distance is that the observed effect of the present study had greater external validity. We experienced that direct personal contact to school pupils was the most efficient way to recruit participants.

Exposure in the intervention group

In the field test children bicycled to school at a self-chosen average intensity of 73.6% of maximal heart rate, which is similar to Finnish adults who commute at a relative intensity of approximately 73.5-78.6%[26]. The relative intensities for adult commuting were converted from %VO_2peak to %maximal heart rate by means of the regression equation reported by Swain[27]. Interestingly, web-assessed distance to school was 2.27 km (SD = 1.55) in the intervention group, whereas GPS-assessed distance in the same group was 2.13 km (SD = 1.51) (p = 0.08). Thus, though needed to be investigated further, self-reported commuter distance in children seem to be valid with (SD delta variable = 0.34).

Despite short school travelling distance, short study duration and non-perfect compliance among the participants included in our ITT-analyses, a significantly decrease in sum of Z score was found in the intervention group compared to the control group. Accelerometer measurements conducted midway in the study indicated that this decrease was not mediated through an increased level of general physical activity since neither mean count nor adjusted minutes at various intensity levels (the latter not included in the results section) were statistically significant different between groups. Our rationale for including children walking to school was based on previous studies reporting no significant differences in various measures of physical fitness between participants walking to school and those using passive transport[8,23].

Main message

Though comparison of effect sizes should be done with caution[28] a lowering of the composite Z score by 0.58 is substantial. In comparison an increase in continuous metabolic syndrome score of 1 has been associated with an odds ratio of developing type-2 diabetes mellitus of 3.4 and 5.1 in men and women, respectively, and odds ratio for cardiovascular disease of 1.7[29]. Consequently a lowering of metabolic syndrome score by 0.58 could theoretically lower the odds of developing diabetes about 45% and CVD around 25%. In conclusion bicycling to school counteracted a clustering of cardiometabolic risk factors and should thus be recognized as potential prevention of type 2 diabetes mellitus and cardiovascular disease.

Figure legends:

Figure 1. Participants flow diagram

Figure 2. Dose-response between bicycling to school and fitness improvement in the intervention group.

Acknowledgments

We thank the participating children and parents and the Biomedical Laboratory Scientists.

Contributors

LØ designed the study, performed the randomisation, analysed and interpreted data, drafted the manuscript, and is guarantor. JT collected, analysed and interpreted the data and revised the manuscipt. LAB revised the manuscript. LBA interpreted data and revised the manuscript. All authors approved the submitted version of the manuscript.

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Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work.

Ethical approval

The study was approved by the regional Ethics Committee. Written informed consent was obtained from the child's parent or legal guardian.

Data sharing statement

Data will not be publically accessible. However, interested individuals may contact the corresponding author.

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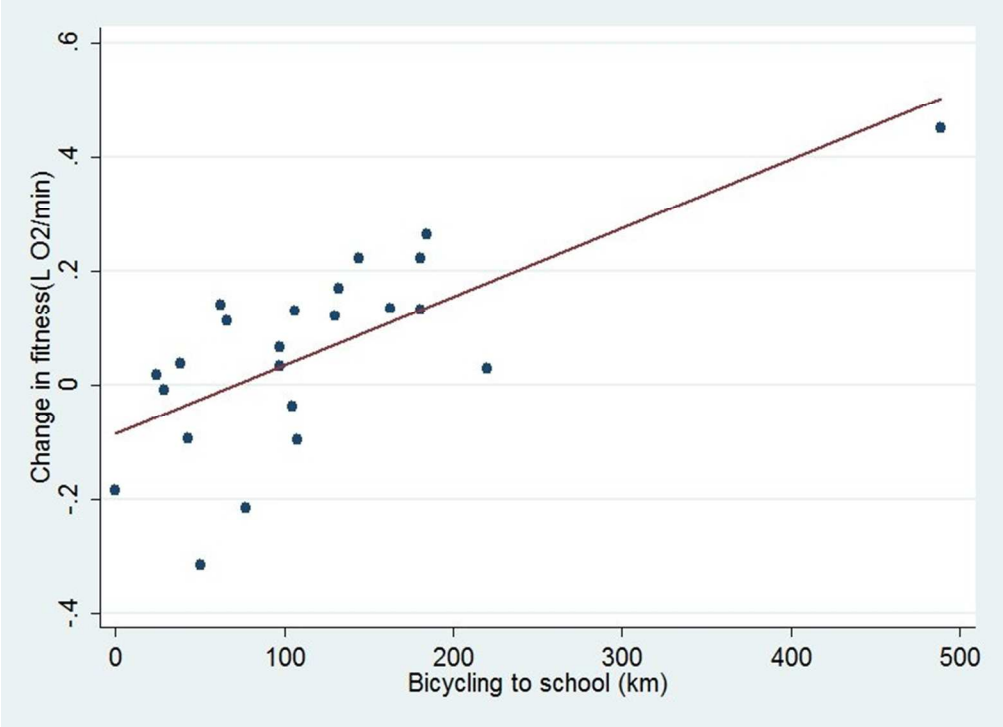

Figure 2. Dose-response between bicycling to school and fitness improvement in the intervention group
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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	13
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5
	6b	Any changes to trial outcomes after the trial commenced, with reasons	
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	15
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5+12

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	5+figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	5
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	12
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	10
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	15

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

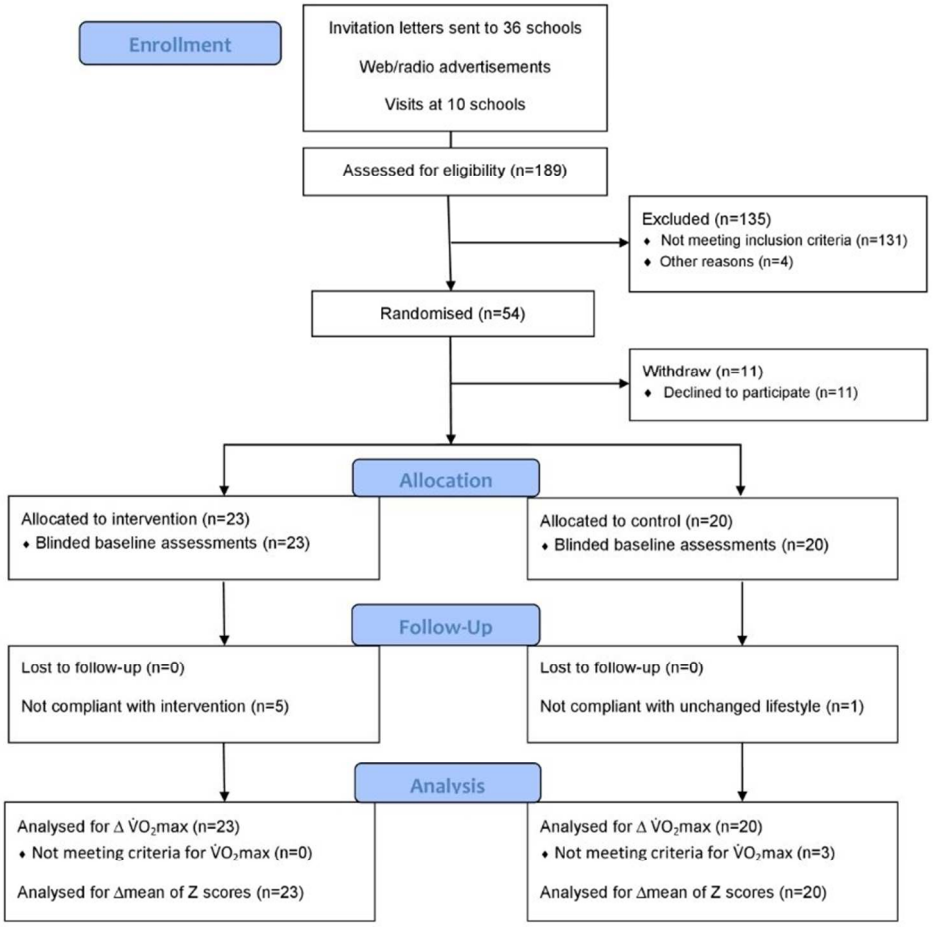

Figure 1. Participants flow diagram

TITLE PAGE

Bicycling to school improves the cardiometabolic risk factor profile: a randomised controlled trial

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Keywords: cardiometabolic, risk factors, bicycling, children, commuting

Word count: Abstract: 199, Main text: 4.343

ABSTRACT

Objectives:

To investigate whether bicycling to school improve cardiometabolic risk factor profile and cardiorespiratory fitness among children.

Design:

Prospective, blinded, randomised controlled trial.

Setting:

Single centre study in Odense, Denmark.

Participants:

43 children previously not bicycling to school were randomly allocated to control group (n=20) (i.e. no change in lifestyle) or intervention group (i.e. bicycling to school) (n=23).

Primary and secondary outcome measures:

Change in cardiometabolic risk factor score and change in cardiorespiratory fitness.

Results:

All participants measured at baseline returned at follow-up. Based upon intention to treat analyses, clustering of cardiometabolic risk factors was lowered by 0.58 SD (95% CI -1.03 to -0.14, p= 0.012) in the bicycling group compared to the control group. Cardiorespiratory fitness (L $O_2 \cdot min^{-1}$) per se did not increase significantly more in the intervention than in the control group (beta = 0.0337, 95% CI -0.06 to 0.12, p=0.458).

Conclusions:

Bicycling to school counteracted a clustering of cardiometabolic risk factors and should thus be recognized as potential prevention of type 2 diabetes mellitus and cardiovascular disease. The intervention did, however, not elicit a larger increase in cardiorespiratory fitness in the intervention group as compared to the control group.

Trial registration:

Registered at www.clinicaltrials.gov (NCT01236222)

ARTICLE SUMMARY

Article focus

- Intervention studies investigating whether bicycling to school in fact cause improved cardiometabolic health have not yet been carried out.
- The aim of this study was to investigate whether bicycling to school cause an increase in cardiorespiratory fitness and induce improvement of clustering of cardiometabolic risk factors

Key messages

- This study is the first study to document that bicycling to school had positive effect on clustering of cardiometabolic risk factors in children.

Strengths and Limitations

- The major strength of this study was the randomised design which through elimination of bias allowed for investigation of a causal relationship between bicycling to school and health outcomes.
- Relatively weak statistical power and impossibility of blindness of the treatment.

INTRODUCTION

The metabolic syndrome (MS) is the concurrence of multiple risk factors associated with cardiovascular disease and type 2 diabetes mellitus. Prevalence is between 20-30% of the adult population in most countries[1], and a rising incidence in children and adolescents[2], indicates that the condition represents a major threat to global public health. Exposure of the MS confers a doubled risk of incident cardiovascular event and death[3] and up to 5-times higher risk of developing type 2 diabetes mellitus in adults[4]. Furthermore, since clustering of cardiometabolic risk factors track from childhood to adulthood[5] early prevention of the MS is important in order to prevent occurrence of disease later in life.

In children and adolescents physical activity has the potential to prevent a clustering of risk factors[6], underpinned by the notion that an increase of daily moderate physical activity by 10-20% is associated with 33% lower risk of having the MS[7].

Active commuting such as walking and bicycling to school might be important contributors of preventive physical activity. Observational studies indicate that bicycling to school is beneficial to health since higher cardiorespiratory fitness[8] and lower BMI[9] have been observed in children bicycling to school compared to non-cyclists. If bicycling to school is generally acknowledged as a time-efficient and feasible form of daily physical activity it could potentially target the entire population. From a public health perspective this would be very appealing considering that the greatest health benefits are achieved in the least active individuals [10].

There seems to be wide-ranging scepticism about the opportunity to bicycle to school in industrialized societies. The level of concern, however seems often to be negatively associated with the prevalence of bicycling to school, which in Denmark for instance is about 60% among adolescents [8,9]whereas in the UK approximately 2% bicycle to school [11].

Intervention studies investigating whether bicycling to school in fact cause improved cardiometabolic health have not yet been carried out. One reason for this could be that very few countries have an infra-structure allowing safe commuting by bike to school. Another reason could be that interventions often include change in the built environment in order to provide safe routes and this is difficult to control in a rigid study design.

The evidence linking bicycle commuting with cardiometabolic health in children is still limited and experimental studies investigating causality have been requested [12]. Hence, the aim of this study is to investigate, in a randomised trial, whether bicycling to school cause an increase in cardiorespiratory fitness and induce improvement of clustering of cardiometabolic risk factors.

METHODS

Study participants

This study took place in Denmark in the municipality of Odense during spring 2011. Participants were recruited through invitation letters sent to 36 elementary public schools in the municipality of Odense. Investigators then visited 10 of these schools (approximately 92 classes) and presented the project. Additionally, the project was presented online and in a radio spot. Participants were included if they at the time of registration stated that they had not bicycled regularly to and from school for at least 3 months (i.e. at least from January onwards) prior to the intervention, and if willing to be randomised to one of the two study groups (i.e. control group or bicycling group). Reasons for exclusion were not having a bike, and/or affirming less than one km between home and school. One hundred and eighty-nine children volunteered to participate in the study. One hundred and thirty-one of these, however, claimed that they bicycled regularly to school, and four withdrew from the study due to a changed family situation. Fifty-four eligible children were subsequently randomised to either control or intervention. After randomisation six and five participants from control and intervention group, respectively, withdrew since they did not accept being randomized, leaving 43 participants in the study (Figure 1). Written informed consent was obtained from the child's parent or legal guardian after they were given a detailed written explanation of the aims of the study, possible hazards, discomfort, and inconvenience, and the option to withdraw at any time. The study was approved by the regional Ethics Committee (S-20100009) and registered at clinicaltrials.gov (NCT01236222) with the purpose of investigating the effect of bicycling to school on the metabolic syndrome and aerobic fitness.

Study design and intervention

Participants were randomly assigned to a control group (i.e. no change in lifestyle) or to an experimental group (i.e. commuter bicycling). A permuted random block size (2, 4 and 6) randomisation (www.randomization.com) was used in order to reduce the chances of the assignment schedule being seen by those responsible for recruitment of participants[13]. Twenty participants were allocated to the control group and 23 participants to the bicycling group. All measurements were repeated at the conclusion of the 8-week intervention program. There were no restrictions beside the mode of transportation to school. The study period comprised one week of national holiday and additionally two bank holidays where no children attended school. Matching baseline test date with follow-up test (in order to achieve similar intervention duration between subjects) was strived for, but logistically not possible for all participants. This has implications on the amount of exposure the intervention group was able to accumulate. Participants could maximally accumulate between 56 and 74 trips to and from school during

the study period. All measurements at baseline and follow-up were conducted by personal blinded to group allocation. Children were picked up and returned to their home addresses when scheduled for baseline and follow-up tests.

Measurements

Height was measured by a stadiometer (SECA, Hamburg, Germany), children wearing socks or bare feet and weight was assessed by a 0.1 kg precision scale (Soehnle Professional, Murrhardt, Germany) wearing only shorts and t-shirts. Skinfold thickness was measured on the left side with a Harpenden caliper (John Bull, British Indicators Ltd., West Sussex, England) at the biceps, triceps, subcapsular, and suprailiac sites. Overweight/obesity status was defined according to age- and gender specific published cut-points for BMI[14].

Systolic and diastolic blood pressure was measured by a sphygmomanometer (DINAMAP ProCare 100, General electric, USA). At least five measurements were made on the left arm with two min interval and the mean of the final three measurements were used as systolic and diastolic pressure.

All Children were instructed to fast overnight (>8 hours), and only allowed to drink water until the blood sample was drawn. Blood was drawn from the right fossa cubitus by a biomedical laboratory scientist. If a participant had not been fasting or if he/she had experienced any illness during the last week, a new test day was scheduled for that child. Samples were analysed for: Glucose, insulin, cholesterol, triglyceride. Insulin resistance was estimated according to the homoeostasis model assessment (HOMA) as the product of fasting glucose (mmol/L) and insulin (µU/mL) divided by the constant 22.5[15]. Breakfast was served on site subsequent of blood sampling. All analysis of the blood specimens were conducted at Department of Clinical Biochemistry and Pharmacology, Odense Universityhospital

Aerobic fitness ($\dot{V}O_2$peak) was determined in a progressive bicycle test with on an electronically braked ergometer (Monark 839 Ergomedic, Varberg, Sweden). Pulmonary gas exchange was measured with a metabolic cart (Amis2001, Innovision, Denmark) with 15 seconds epoch, and the highest value within 30 seconds was regarded as a maximal if respiratory exchange ratio (RER) >= 0.99 or maximal heart rate (HR) was >=185 beats/min, and the test leader judged the participant to show signs of intense effort (e.g. facial flushing or difficulties in keeping up the pedal frequency)[16]. HR was measured with a HRM (Polar RS800CX, Kempele, Finland) with epoch set to 1 sec. Children were thoroughly explained the purpose of the test and that it would require a maximal effort. After a warm-up period of 5 minutes at 40W, work-load was increased 40W every 2 minutes until volitional exertion. Verbal encouragement was given throughout the test and recommended pedaling cadence was 60-80 rpm.

Children completed a general questionnaire regarding transportation to school, sports-habits, and general quality of life. At follow up all children marked their route to school using a web-based map tool (www.loebererute.dk).

All children had their bike equipped with an odometer on average two weeks before baseline measurement. The odometers were individually calibrated in accordance to wheel circumference. Odometer levels were self-reported, by participants in both groups, every Sunday until tested at follow-up by means of a commercial SMS system (SMS-Track ApS). Participants who did not answer Sunday were contacted Monday. Malfunctioning odometers were replaced within a few days.

Both groups were instructed to report daily mode of school transportation on a custom made transport diary. Total mileage of school related bicycling during the study was calculated from the distance to school (web route assessment) times the number of trips to/from school (transport diary).

Field measurent of intensity of bicycling to school was carried out midway (five weeks after baseline). Participants in the intervention group received a GPS (Qstarz BT-Q1300S, Qstarz International Inc., Taiwan) and a heart rate monitor (HRM) (PolarTeam^2, Polar, Kempele, Finland) and were instructed to wear the devices during one day. Both GPS and HRM were set at an epoch of 5 second. All GPS data were transferred to commercial software (Travel recorder v. 5.0) and corrected for drift using manufacturer software. The GPS was the reference for all HRM data. Mean HR of school transportation, if verified by the diary, was calculated as the mean of the measurements from the first data point when the child exceeded a speed of 5 km/h when leaving home, to the last data point above 5 km/h when the child arrived at school. Both compliant and non-compliant participants were included in the determination of commuter bicycling intensity since the primary outcome is based on intention to treat (ITT) analyses.

Physical activity beyond bicycling was assessed with an accelerometer (Actigraph GT3X, Manufacturing Technology Inc, Fort Walton Beach, FL, USA). Physical activity was monitored for 7 consecutive days from Monday to Sunday at baseline and midway (five weeks after baseline). Instruments were attached at the hip, and counts were sampled every two seconds. Data reduction was performed with customised software (Propero, Odense, Denmark). Only data from the vertical axis was included in the data analyses. Criteria for a successful recording were a minimum of 3 days of 10 h recording per day. Time periods of at least 30 consecutive minutes of zero counts were deemed to represent periods when the monitor was not worn and thus disregarded. Cut points for intensity levels were based on the Freedson/Trost equation[17]. Since cutpoints for physical activity intensity are specifically designed for 1 min epoch these were divided by 30. The average time the accelerometer was worn was 13.5 and 13.6 h per day at baseline and follow up, respectively, and the number of minutes spent in the different intensity

intervals was proportionally adjusted to 14 h with the following equation: adjusted minutes=(observed in interval)×(14×60/total minutes) [18].

Values for all blood parameters at both baseline and follow-up are missing in one participant from the intervention group due to needle phobia. Insulin and HOMA values were regarded as missing for one participant in the control group due to type 1-diabetes. Follow up cholesterol for one person and baseline HOMA for another participant from the intervention group were not obtained due to irregularity at the laboratory. Systolic blood pressure is missing in one participant due to resistance.

One participant from the intervention group performed maximal tests, but was not measured with the metabolic cart. $\dot{V}O_2$peak was in this case estimated from the regression equation between power output (MPO) and $\dot{V}O_2$peak of the study sample. Change in $\dot{V}O_2$peak was considered missing in 3 participants since test criteria was not met at follow-up.

Statistics

Crude baseline measurements were compared between the bicycling and the control group participants using unpaired mean comparison tests (t-tests), whereas paired t-tests were used in within group comparisons from baseline to follow-up.

Post intervention values were analysed across the two groups using analysis of co-variance (ANCOVA), as suggested by Twisk and Proper[19], with participants grouped as originally randomised regardless of the degree of intervention compliance and types of activity actually performed. In efficacy analyses participants are referred to as compliant in the intervention group if at least 80% of possible trips to school were by bike on contrast participants in the control group are considered compliant if less than 20% of possible trips were by bike. Change in outcome was compared between the bicycling and the control group with adjustment for baseline measure and gender. All covariates were selected a priori and thus in any case kept in the statistical modeling even if non-significant. Regression analyses were preceded by verification of fulfillment of parametric assumptions by qq-plots and Shapiro Wilks tests.

Z scores (observed value - baseline mean / baseline SD) were computed for each of the variables included in the composite Z score. A high Z score value (or an increase) is considered to have adverse health effects for all variables included in the composite score with exception of cardiorespiratory fitness where inverse values were calculated. Composite Z scores, based on baseline and follow-up measurements, were constructed as a mean of the available standardised selected risk factors (fasting triglyceride, insulin sensitivity (HOMA), sum of four skinfolds, systolic blood pressure, total cholesterol/HDL ratio and inverse aerobic fitness). Composite baseline and follow-up Z scores were then standardised according to the baseline mean and SD allowing for interpretation of change in composite Z score. Subsequently the change

in standardised composite Z score was calculated as (standardised mean of Z scores at follow up - standardised mean of Z scores at baseline).

Assuming a mean change of 10% in $\dot{V}O_2$peak and a SD of change of 0.3 L $O_2 \cdot min^{-1}$ the study needed 21 participants in both groups to be powered at 80%. All analyses were conducted using STATA IC version 11.0 (STATA Corp, College station) with alpha=0.05.

RESULTS

Background characteristics

Of the 54 participants randomized, 11 declined to participate (two due to moving, one due to personal reasons, one due to parental job situation, two due to test methods and five not accepting randomisation) leaving 43 participants (79.6%) in the study. No subjects experienced accidents or other harms. All 43 participants were measured at baseline and returned for follow-up (Fig. 1). Based on published age and gender BMI cut-off values [14] no participants were obese at baseline, whereas three and two participants were overweight in the bicycling and control group, respectively. There were no statistical differences between the control group and the bicycling group participants who completed the 8-week intervention period on any of the baseline features presented in Table 1, nor was there any baseline difference in the two primary dependent variables: $\dot{V}O_2$peak and mean of Z scores.

(insert table 1 here)

Adherence

All of the 43 allocated participants were available at follow-up assessments. Five participants in the intervention group and one in the control group were defined as non-compliant. The average compliance was 96.2% and 84.2 % in the control and the intervention group, respectively.

(insert table 2 here)

(insert table 3 here)

Change in $\dot{V}O_2$peak from baseline to follow-up

Crude comparisons of the effects within groups showed that $\dot{V}O_2$peak mean increased from 1.81 at baseline to 1.87 L $O_2 \cdot min^{-1}$ at follow-up (i.e. an increase by 3.3%) in the intervention group whereas the change in the control group was 1.69 vs. 1.73 L $O_2 \cdot min^{-1}$ (i.e. an increase by 2.3%). Based on adjusted ITT regression analyses children who started bicycling to school did not improve fitness (beta = 0.0336911, p= 0.458) compared to controls. Likewise based on adjusted estimates from efficacy analyses children who started bicycling to school did not improve fitness (beta = 0.0425191, p= 0.404) compared to controls. There was a significant association (p<0.001) between kilometers bicycled to school and fitness improvement (figure 2).

Change in composite Z score from baseline to follow-up

Crude comparisons of the effects within groups showed that the standardised composite Z score decreased from 0.01 at baseline to -0.26 at follow-up in the intervention group, whereas in the control group the corresponding change was from 0.01 to 0.28.

Based on adjusted ITT regression analyses children who started bicycling to school lowered their composite standardised Z score (beta = -0.58, p=0.012) compared to controls. Based on adjusted efficacy analyses children who started bicycling to school lowered their standardised composite Z score (beta = -0.63, p=0.015) compared to controls. There was no significant association (p=0.512) between kilometers bicycled to school and change in composite Z score.

Post hoc regression analyses of the 6 Z transformed delta variables included in the composite score showed that every single variable, although statistically non-significant individually, contributed to the lower standardised composite score in the intervention group. Standardised delta coefficients were: Inverse fitness -0.085867 (p= 0.458), systolic blood pressure -0.1074832 (p=0.644), sum of 4 skinfolds -0.1522508 (p=0.118), triglyceride -0.2713089 (p=0.295), cholesterol ratio -0.0405599 (p=0.838), HOMA score -0.4582449 (p=0.049).

(insert table 4 here)

DISCUSSION

Statement of principal findings

The main finding in this study was that bicycling to school for a period of 8 weeks significantly lowered clustering of cardiometabolic risk factors by 0.58 SD. The effect size estimate was different between the control and the intervention group in ITT analyses, though not all participants were fully compliant during the study period. The level of statistical significance of the clustered risk score was statistically significant even when multiple testing (due to two outcomes) was taken into account with the conservative Bonferroni method [20].

Standardized residuals were plotted against the predicted values and no systematic patterns were observed which confirmed variance-homogeneity. QQ plots and Shapiro wilks tests of the standardized residuals of the model expressed normality. The goodness of fit as indicated by r-squared values in the regression modeling of change in the standardised composite Z score were 0.16 and 0.19 for ITT and efficacy analyses respectively. Goodness of fit for the modeling of change in $\dot{V}O_2$peak was 0.17 and 0.18 for ITT and efficacy analyses respectively (for additional results from the regression analyses see supplement 2).

Post hoc regression analyses of the 6 Z transformed delta variables included in the composite score showed that every included variable contributed to the improved clustered score. This is noteworthy since it indicates a consistent positive effect of cycling to school on a range of various health parameters.

The composite risk factor score was used in order to compensate for the day-to-day fluctuation in the single risk factors and included the same variables as in previous studies by our group[6]. A continuous score as a proxy of the metabolic syndrome was chosen in favor of a dichotomous outcome in order to enhance statistical sensitivity, and because it is considered to describe the metabolic health condition better [21]. Further, none of the children had MS according to the definition by the International Diabetes Federation [22].

Bicycling to school did not have the expected effect on cardiorespiratory fitness per se since no significant difference between cyclists and controls was observed. This was contra intuitive since 4.6-5.9 % higher aerobic power has been reported in cross sectional studies comparing adolescents bicycling to school to walkers and passive transporters [23]. Furthermore a 9 % improvement has been found in children who started bicycling to school in prospective studies by Cooper et al. [24]. The discrepancy might be due to a relatively short intervention period in the present study and perhaps also because of too short distance to school. The latter being indirectly indicated from the significant association between kilometers bicycled to

school and cardiorespiratory fitness improvement. In this connection it is noteworthy that a 7.3% improvement in $\dot{V}O_2$max [25] has been observed in a randomised study of adults who started bicycling to work and that the commuting trip on average was about 5km, which is twice the distance of the children included in this study. Interestingly post-hoc linear regression showed that both the relative average and the relative maximal intensity during commuter bicycling was positively associated (see data supplement 1) with cardiorespiratory fitness improvements (p=0.005 and p=0.002 respectively).

Finally, we cannot rule out that the non-significant difference in changes of $\dot{V}O_2$peak between groups could be a consequence of lack of statistical power (i.e. type II-error) since a change in $\dot{V}O_2$peak by 10% would require 21 perfectly compliant participants in both groups to be powered at 80%. The preliminary power calculations in the present study were based on expected change in $\dot{V}$O2peak since no previous data on the potential effect of bicycling to school on cardiometabolic health were available.

Compliance

Participants did not exhibit a behavior that was fully in accordance to the group they were randomised into. During the intervention period five participants in the intervention group bicycled less than 80% of all possible trips to school, and one participant in the control group cycled more than 20% of all trips to school by bike. A relative cutoff value of 80% was set allowing room for participants to be included in the efficacy analyses though subject to sickness, bike theft and the like. This arbitrary relative cutoff was preferred to an absolute due to slightly varying study duration and coincides well with the frequency of bicycling observed in the underlying population. In the ITT analyses we maintained the strengths of the RCT-design and accounted for potential known and unknown confounding through inclusion of all allocated participants in the analyses knowing that the true health potential of bicycling to school is likely to be larger than our estimates. In supplementary efficacy analyses we accounted for the non-compliance and found slightly larger effect estimates for both composite Z score and $\dot{V}O_2$peak. We included "non-bicycling" participants based on self-reported mode of transportation to school. From the transport diaries it was possible to assess whether participants in the period preceding the study had in fact been non-bicycling and meeting inclusion criteria (as self-claimed). Four participants in the control group and one in the intervention unfortunately bicycled more than 20% of possible trips to school in the two weeks pre-baseline period. Participants were kept in both ITT and efficacy-analyses though their appearance possibly diluted the true effect of commuter bicycling to school. It is evident that acceptance and understanding of randomisation of transport to school was a challenge. Transport to school is an integrated part of the daily logistics of the entire family making compliance highly susceptible to various factors such as parental job situation, parental marital status and sudden extra vacation.

Strengths and weaknesses of the study

The major strength of this study was the randomised design which through elimination of bias allowed for investigation of a causal relationship between bicycling to school and health outcomes. Compliance was supported by inclusion of participants from numerous classes consequently diminishing the risk of contamination between groups. Direct measurements of $\dot{V}O_2$peak and all baseline and follow-up measurements being carried out by the same experienced, and blinded test personnel are likewise study strengths. Furthermore detailed information on the degree of exposure before (allowing assessment of fulfillment of inclusion criteria) and during (e.g. mode of transportation to school, amount of bicycling to school, amount of bicycling beyond commuter bicycling, commuter bicycling intensity, general physical activity level) the study is advantageous. A study weakness was a relatively small study sample not behaving perfectly in accordance to the group randomised into, and consequently possible compromised the detection of an effect of bicycling to school on cardiorespiratory fitness. It is likely that the amount of total bicycling assessed from SMS-reported odometer status were underestimated because some odometers reset due to malfunction. Possible this underestimation is biased as the intervention group generally bicycled more trips and thus was more susceptible to theft and malfunction. This, however, is not crucial in the present study focusing on commuter bicycling being assessed from the transport diaries. Field measurements of commuter bicycling intensity and distance are based solely on one single trip to school and should therefore be taken with caution. Finally we cannot rule out that the field measurements have been biased due to the Hawthorne effect (i.e. children may have changed their behavior as they knew they were being studied). A possible Hawthorne effect should, however, beside from the field measurements be equal in the two study arms.

Recruitment

It was feasible to carry out the present study because it is relatively safe and feasible to bicycle to school in Denmark. The recruitment of participants, however, turned out to be rather difficult partly due to the fact that approximately 60% of children in the region already bicycled to school[9] and thus not includable. From the remaining 40% some lived too far away from the school to bicycle, while others lived so close that they preferred to continue walking. Others were unable or unwilling to have their daily transport dictated by a randomisation. The recruitment difficulties forced us to include participants living closer to the school than our pre-determined inclusion criteria of 2km. The inference of inclusion of children with short commuter distance is that the observed effect of the present study had greater external validity. We experienced that direct personal contact to school pupils was the most efficient way to recruit participants.

Exposure in the intervention group

In the field test children bicycled to school at a self-chosen average intensity of 73.6% of maximal heart rate, which is similar to Finnish adults who commute at a relative intensity of approximately 73.5-78.6%[26]. The relative intensities for adult commuting were converted from %VO_2peak to %maximal heart rate by means of the regression equation reported by Swain[27]. Interestingly, web-assessed distance to school was 2.27 km (SD = 1.55) in the intervention group, whereas GPS-assessed distance in the same group was 2.13 km (SD = 1.51) ($p = 0.08$). Thus, though needed to be investigated further, self-reported commuter distance in children seem to be valid with (SD delta variable = 0.34).

Despite short school travelling distance, short study duration and non-perfect compliance among the participants included in our ITT-analyses, a significantly decrease in sum of Z score was found in the intervention group compared to the control group. Accelerometer measurements conducted midway in the study indicated that this decrease was not mediated through an increased level of general physical activity since neither mean count nor adjusted minutes at various intensity levels (the latter not included in the results section) were statistically significant different between groups. Our rationale for including children walking to school was based on previous studies reporting no significant differences in various measures of physical fitness between participants walking to school and those using passive transport[8,23].

Main message

Though comparison of effect sizes should be done with caution[28] a lowering of the composite Z score by 0.58 is substantial. In comparison an increase in continuous metabolic syndrome score of 1 has been associated with an odds ratio of developing type-2 diabetes mellitus of 3.4 and 5.1 in men and women, respectively, and odds ratio for cardiovascular disease of 1.7[29]. Consequently a lowering of metabolic syndrome score by 0.58 could theoretically lower the odds of developing diabetes about 45% and CVD around 25%. In conclusion bicycling to school counteracted a clustering of cardiometabolic risk factors and should thus be recognized as potential prevention of type 2 diabetes mellitus and cardiovascular disease.

Figure legends:

Figure 1. Participants flow diagram

Figure 2. Dose-response between bicycling to school and fitness improvement in the intervention group.

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Contributors

LØ designed the study, performed the randomisation, analysed and interpreted data, drafted the manuscript, and is guarantor. JT collected, analysed and interpreted the data and revised the manuscipt. LAB revised the manuscript. LBA interpreted data and revised the manuscript. All authors approved the submitted version of the manuscript.

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Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organisation for the submitted work.

Ethical approval

The study was approved by the regional Ethics Committee. Written informed consent was obtained from the child's parent or legal guardian.

Data sharing statement

Data will not be publically accessible. However, interested individuals may contact the corresponding author.

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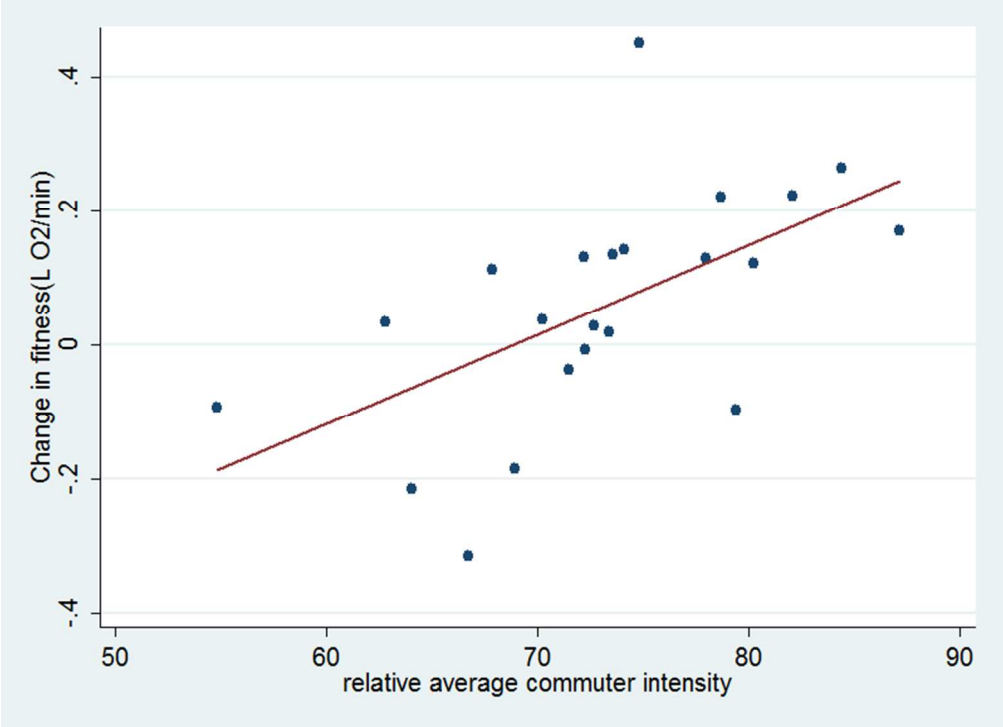
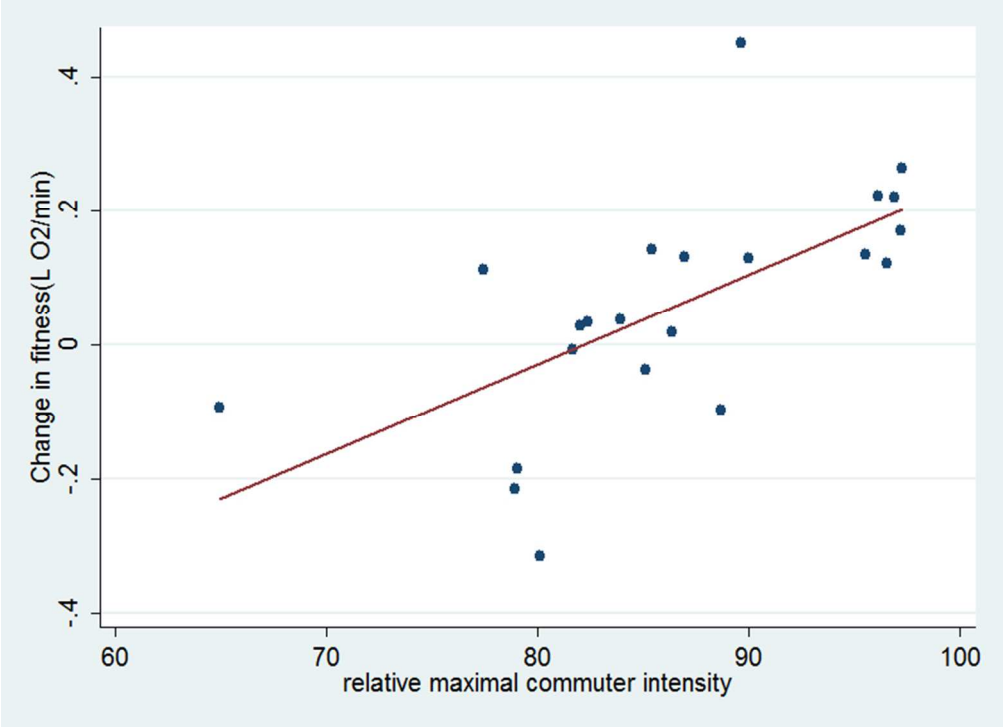


Association between relative average commuter intensity and cardiorespiratory fitness improvements
281x204mm (72 x 72 DPI)



Association between relative maximal commuter intensity and cardiorespiratory fitness improvements
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Data supplement 2. Results from regression analyses

$\dot{V}O_2$peak change (intention to treat)

	Beta	SE	t	P
Baseline VO2 peak	-0.1524	0.0600	-2.54	0.016
Group (intervention)	0.0337	0.0449	0.75	0.458
Gender (female)	-0.0030	0.0475	-0.06	0.950
Constant	0.3008	0.1161	2.59	0.014

$\dot{V}O_2$peak change (per protocol)

	Beta	SE	t	P
Baseline $\dot{V}O_2$peak	-0.1569	0.0642	-2.44	0.021
Group (intervention)	0.0425	0.0502	0.85	0.404
Gender (female)	-0.0111	0.0527	-0.21	0.835
Constant	0.3076	0.1232	2.50	0.018

Composite Z score change (intention to treat)

	Beta	SE	t	P
Baseline composite Z-score	-0.0387	0.1194	-0.32	0.748
Group (intervention)	-0.5838	0.2208	-2.64	0.012
Gender (female)	0.1164	0.2414	0.48	0.632
Constant	0.4129	0.1883	2.19	0.034

Composite Z score change (per protocol)

	Beta	SE	t	P
Baseline composite Z-score	-0.1562	0.1574	-0.99	0.328
Group (intervention)	-0.6282	0.2435	-2.58	0.015
Gender (female)	0.0856	0.2529	0.34	0.737
Constant	0.4253	0.1994	2.13	0.040