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Associations between Bicycling and Carotid Arterial Stiffness in Adolescents: The European Youth Hearts Study

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Running head:	Bicycling and arterial stiffness

Abstract

The aim of the study was to investigate the associations between bicycling and carotid arterial stiffness, independent of objectively measured moderate-and-vigorous physical activity. This cross-sectional study included 375 adolescents (age 15.7± 0.4 years) from the Danish site of the European Youth Heart Study. Total frequency of bicycle usage was assessed by self-report and carotid arterial stiffness was assessed using B-mode ultrasound. After adjusting for pubertal status, body height and objectively measured physical activity and other personal lifestyle and demographic factors, boys using their bicycle every day of the week displayed a higher carotid arterial compliance [Std. beta 0.47 (95% CI 0.07 to 0.87)] and distension [Std. beta 0.38 (95% CI -0.04 to 0.81). Boys using their bicycle every day of the week furthermore displayed a lower Young's Elastic Modulus [Std. beta -0.48 (95% CI -0.91 to -0.06]. Similar trends were observed when investigating the association between commuter bicycling and carotid arterial stiffness. These associations were not observed in girls. Our observations suggest that increasing bicycling in adolescence may be beneficial to carotid arterial health among boys.

Introduction

Early modification of CVD risk factors, such as low physical activity (PA) levels, is of importance to prevent adverse CVD outcomes later in life (Sattelmair, 2011). Several studies have observed an inverse association between PA and metabolic CVD risk factors in childhood and adolescence (Andersen, 2011a; Ried-Larsen, Grontved, Froberg, Ekelund, & Andersen, 2013), and it is thus important to understand the means by which the PA level can be increased early in life. One way of increasing the PA level, is to promote the use of active transportation such as bicycling. Observational studies have reported inverse association of bicycling with all-cause mortality and cardiovascular disease in adults and with overweight, low cardiorespiratory fitness and metabolic CVD risk factors in children and adolescents (Cooper, 2006; Cooper, 2008; Ostergaard, 2012; Andersen, Schnohr, Schroll, & Hein, 2000; Matthews, 2008; Andersen, 2011b; Andersen, 2011a; Cooper, 2006; Ostergaard, Borrestad, Tarp, & Andersen, 2012).

Carotid arterial stiffness has been suggested to be predictive of stroke in adults independent of conventional CVD risk factors (Yang, 2012). The independent predictive value of arterial stiffness suggests an added value beyond conventional CVD risk factors and could thus act as a stronger intermediate CVD endpoint compared to conventional CVD risk markers (Laurent, 2006). Carotid arterial stiffness can be measured non-invasively using ultrasound techniques and could therefore prove useful for the understanding of early CVD detection (Urbina, Khoury, Martin, D'Alessio, & Dolan, 2009). Low PA is associated with adverse arterial health in childhood and adolescence (Abbott, Harkness, & Davies, 2002; Hopkins, 2009; Reed, 2005; Schack-Nielsen, Molgaard, Larsen, Martyn, & Michaelsen, 2005; Stone, Rowlands, Middlebrooke, Jawis, & Eston, 2009; Pahkala, 2008; Sakuragi, 2009; Ried-Larsen, 2013). However, to our knowledge no studies have evaluated the association between bicycling and carotid arterial stiffness independent of other habitual moderate-vigorous PA

level. If providing consistent evidence to support the health benefits of bicycling early in life, independent of engagement in other PA, increasing bicycling could be recognized as an important target for intervention. The potential health benefits of promoting bicycling is large since in many countries only a small proportion of youth use the bicycle as a mean of transportation and during leisure time (Department for transport, 2013). Therefore, the aim of the study was to examine whether there is an association between the frequency of bicycle use and carotid arterial stiffness independent of other moderate-and-vigorous physical activities in a population-based sample of adolescent boys and girls.

Material and methods

Participants and design

This was a cross-sectional study using data from the Danish site of the European Youth Heart Study (EYHS). EYHS is an international population-based mixed longitudinal study that addresses biological, environmental, demographic and lifestyle correlates and correlates of cardiovascular disease risk factors in children and adolescents. A description of the EYHS protocol and the sampling procedures has been reported elsewhere (Riddoch, 2005). Ultrasonography was added to the protocol in 2009-2010 when a random sample of 709 fifteen-year old children was invited to take part in the study. Participation rate was 59% (N= 399) and the present study reports on 375 participants with complete data on exposure and outcome. The study was approved by the Regional Scientific Ethical Committee for Southern Denmark and data were collected according the Helsinki declaration. All participants gave a written informed consent.

Bicycling

The frequency of total bicycle (including commuter bicycling) use and travel mode when commuting was determined using a computerized questionnaire including the question; “How often do you ride your bike?”. The response options were; **1)** “Hardly or never”, **2)** “One or two time a week”, **3)** “Nearly every day” or **4)** “Every day”. As there were very few respondents in categories one (boys/girls: 21/15) and two (boys/girls: 22/29) these were combined into one category; “Less than three times a week” in order to preserve statistical power. Information on mode of commuting to school was obtained from the question: "How do you usually travel to school?" with the response options: 1) by car or motorcycle, 2) by bus or train, 3) by bicycle or 4) by foot. To preserve statistical power, the response options one and two were grouped into the category; passive transport.

Carotid arterial properties

The carotid arterial properties were measured using ultrasonography (Model Logic e, 12L-RS probe (5-13 MHz, 12 MHz used) GE Medical) according to guidelines (Touboul, 2007; Laurent, 2006). The arterial properties were recorded at the lateral and posterior position of the common carotid artery, 10 mm and 20 mm (for arterial stiffness measures) proximal to the beginning of the carotid bulb on both the right and the left common carotid artery. Carotid IMT (cIMT) was obtained at the far wall of the artery. Prior to the assessments, the participants rested in a supine position for 10 minutes in a quiet temperature controlled room.

Images from eight cardiac cycles were stored offline for quantification of carotid artery diameters and the cIMT. The analyses were performed by a blinded trained reader, using commercially available analysis software (Vascular Research Tools 5, Medical Imaging Applications, LLC). Peak-

systolic (DS), end-diastolic (DD) arterial diameter and cIMT were obtained from both positions. The mean of both positions and both sides was used in the subsequent analysis.

Brachial systolic and diastolic blood pressure (BP) were obtained from the right arm at the end of the examination in a supine position (Welch Allyn Vital Signs monitor 300 series, Kivex, Hoersholm Denmark) using an appropriate cuff size. Brachial pulse pressure (PP) was calculated as systolic minus diastolic BP. All examinations were performed by a single trained operator. Intra-reader coefficients of variation were 5.7 %, 4.5% and 4.5% for cIMT, systolic and diastolic diameter, respectively.

The compliance coefficient (CC), the distensibility coefficient (DC), and Young's elastic modulus (YEM) were calculated as follows (van de Laar, 2010; Yang, 2012);

1) $\mathrm{CC} = \pi * (\mathrm{DS}^2 - \mathrm{DD}^2)/(4 * \mathrm{PP})$ in $\mathrm{mm}^2/\mathrm{kPa}$
2) $\mathrm{DC} = (2*(\mathrm{DS}-\mathrm{DD})*\mathrm{DD}+(\mathrm{DS}-\mathrm{DD})^2)/(\mathrm{PP}*\mathrm{DD}^2)$ in $10^{-3}/\mathrm{kPa}$
3) $\mathrm{YEM} = \mathrm{DD}/(\mathrm{cIMT} * \mathrm{DC})$ in 10^3*kPa

For YEM (intrinsic wall stiffness) higher values mean stiffer carotid arteries. For CC (buffering capacity) or DC (elastic properties of the artery) higher values means lower stiffness of the carotid arteries.

Blood pressure

Resting brachial BP was assessed with a paediatric and adult neonatal vital signs monitor (Dinamap model XL; Critikron, Tampa, FL, USA). Five measurements were taken at 2-minute intervals. The

mean of the final three measurements were used in the analyses. Prior to measurements individuals rested for 5 minutes while seated. Mean arterial pressure was calculated as diastolic BP*((systolic BP-diastolic BP)/3).

Physical activity

Moderate-and-vigorous PA (expressed as minutes per day) was assessed objectively using the Actigraph GT3X accelerometer-based activity monitor (using 10 second epochs). The moderate-and-vigorous PA cut-point was set to 500 count/10 seconds (3000 counts per minute) (Freedson, Pober, & Janz, 2005). Data reduction and post processing procedures have been described in detail elsewhere (Ried-Larsen, 2013).

Other covariates

Body height, weight and waist circumference were assessed using standard anthropometric procedures were employed. These have been described in detail elsewhere (Andersen, 2006; Riddoch, 2005). Information on soft drink, fruit and vegetable intake (servings/week), TV-viewing and smoking status (yes/no) were obtained using a computerized questionnaire (Riddoch, 2005; Grontved, 2012). Family history of CVD (paternal and maternal) (yes/no) and paternal and maternal educational level were obtained using self-report by the parents. Parental educational level was defined according to the International Standard Classification of Education (ISCED-A) (UNESCO 2011). As the details obtained of the description of education were insufficient to accurately classify the educational level, the ISCED level 0, 1 and 2; 3, 4 and 5, and 6, 7 and 8 were grouped. The highest parental educational level of the mother or father was used in the analysis. Biological maturity was assessed subjectively according to Tanner's classification (Malina, Bouchard, & Bar-Or, 2004).

Statistics

Associations between the frequency of bicycle use and measures of Carotid arterial stiffness were analyzed using analysis of co-variance with group differences presented as standardized values (95% confidence intervals). The statistical models were adjusted for biological development, body height and moderate-and-vigorous PA (model 1). The previous reported independent association between moderate-and-vigorous PA and carotid arterial stiffness (Ried-Larsen, 2013) stress the importance of adjusting for moderate-and-vigorous PA. Since bicycling is almost completely underestimated (app.by 97%, unpublished data) when using waist-worn accelerometers, it was possible to adjust for overall accelerometer-based moderate-and-vigorous PA. Then we included other personal- and lifestyle factors (current smoking status, fruit, vegetable and soft drink consumption, parental educational level, TV-viewing and family history of cardiovascular disease) (model 2). We then included waist circumference as a measure of central adiposity or mean arterial pressure as they may confound or mediate the associations between bicycling and arterial stiffness (Sakuragi, 2009; van de Laar, 2010; Andersen, 2011a; Cooper, 2008). Due to modification by sex ($p<0.1$ for interaction) the analyses are presented in strata of sex. As we have previously published data on commuter mode and CVD risk in children we were interested in the contribution of commuter mode to arterial stiffness (Andersen, 2011a; Cooper, 2008; Ostergaard, 2012; Ostergaard, 2012). Therefore this was investigated secondarily. Due to the low number of passive commuters (N boys=16 and girls=20), thus low power, the analysis were adjusted for body height only.

Information on PA was missing for 50 participants (12.5% of the sample). We therefore imputed the missing values for these individuals using the multiple linear imputation approach ("mi impute" in STATA) including all covariates from model 2. Beta coefficients and the standard errors

from 30 datasets were used. The estimates were adjusted for missing-data uncertainty. Participants with imputed data did not differ from the participants with full PA data regarding age, sex distribution, BMI or frequency of bicycle use (full PA data set) (data not shown).

As the study was observational and due to the explorative nature of the study, corrections for multiple testing were not performed (Bender & Lange, 2001). Standard checks of model assumptions were performed. All statistical analyses were performed in STATA 11.2 (STATA Corp. Fort Valton TX) with alpha=0.05 (two-sided).

Results

The characteristics of the included sample are described in Table 1 (N=375). Neither drop-outs, nor subjects with incomplete data on exposures or outcomes differed from the included sample in terms of BMI, smoking status, biological development or parental education at the baseline assessment performed six years earlier (data not shown). Objectively measured moderate-and-vigorous PA was significantly lower in boys, but not in girls, who reported a higher frequency of bicycle use. Boys had higher carotid compliance, distension and lower values of Young's elastic modules compared to girls ($p<0.05$ for gender differences).

Table 1: Population characteristics among Danish adolescents from the European Youth Heart Study.

	Frequency of bicycle use (boys)				Frequency of bicycle use (girls)			
	<3 times/week	*Nearly every day*	*Everyday*	*P*	*<3 times/week*	*Nearly every day*	*Everyday*	P
N	50	33	86		46	45	115	
Age	15.9 (0.4)	15.6 (0.4)	15.6 (0.4)	<0.001	15.7 (0.4)	15.6 (0.4)	15.9 (0.4)	0.17
Parental education (%low/%middle/%high) †	5/38/57	7/45/48	6/29/65	0.57	13/50/37	7/38/55	5/32/63	0.57
Familial history of CVD (%no/yes)	88/12	94/6	96/4	0.16	82/18	91/9	90/10	0.16
Current smoker (% no/yes)	86/14	90/10	90/10	0.80	89/11	96/4	84/16	0.80
Tanner stage (%3/%4/%5)	14/63/23	23/71/6	22/61/17	0.34				0.43
Sugar sweetened drinks (servings/week)	3.6 (2.5)	3.9 (2.3)	3.9 (2.6)	0.70	3.9 (2.5)	3.6 (2.3)	4.3 (2.5)	0.31
Fruit- and vegetables (servings/week)	12.3 (6.6)	13.3 (5.7)	13.3 (6.7)	0.62	12.5 (6.5)	12.7 (5.8)	14.4 (6.3)	0.11
Moderate-and-vigorous physical activity (min/day)	49 (25)	42 (14)	33 (17)	<0.001	26 (12)	23 (14)	27 (15)	0.29
Carotid compliance (mm^3*kPa^{-1})	1.48 (0.36)	1.63 (0.24)	1.65 (0.41)	0.04	1.48 (0.29)	1.43 (0.26)	1.40 (0.28)	0.29
Carotid distensibility ($\%*kPa^{-1}$)	49.9 (13.0)	53.2 (9.6)	54.4 (13.8)	0.14	52.2 (10.7)	52.0 (10.1)	49.6 (10.0)	0.23
Young's elastic modules ($kPa*mm^{-1}$)	0.24 (0.07)	0.21 (0.05)	0.21 (0.05)	0.08	0.22 (0.05)	0.21 (0.05)	0.23 (0.05)	0.40
Diastolic diameter	6.2 (0.4)	6.2 (0.4)	6.2 (0.4)	0.61	6.0 (0.3)	5.9 (0.3)	6.0 (0.3)	0.38
Pulse pressure (mmHg)	57.1 (10.1)	52.8 (8.6)	53.7 (8.5)	0.05	47.8 (7.5)	49.1 (9.1)	48.8 (6.8)	0.67
Mean arterial pressure (mmHg)	79.7 (7.6)	76.7 (6.1)	76.7 (7.5)	0.06	79.8 (6.6)	77.2 (5.7)	77.6 (6.5)	0.09
Systolic BP (mmHg)	119.3 (11.7)	114.1 (10.2)	116.8 (12.4)	0.17	110.4 (11.4)	111.9 (10.0)	108.5 (7.9)	0.10
Diastolic BP (mmHg)	62.1 (7.9)	59.7 (6.5)	60.2 (6.2)	0.24	63.9 (6.5)	63.6 (6.1)	62.3 (6.0)	0.22
Waist circumference (cm)	76.6 (11.5)	72.9 (6.1)	71.6 (6.6)	0.004	73.3 (9.1)	70.8 (8.1)	71.1 (6.6)	0.004
Body Mass Index (kg/m^2)	21.9 (3.7)	20.4 (2.7)	20.1 (2.4)	0.002	21.9 (3.9)	20.6 (4.6)	21.3 (2.9)	0.002

Data is mean (SD), frequencies (%) or median (interquartile range), p-value is for trend across categories of bicycle frequency

Associations between total bicycling and carotid arterial stiffness

The associations between the frequency of bicycle use and carotid arterial properties and function are described in Table 2. In boys, participants using the bicycle every day of the week had higher values (~0.4 SD) of the carotid compliance coefficient, distensibility coefficient and lower values (~ -0.4 SD) of Young's elastic modules in the crude models (Table 1, model 1), when compared to those using the bicycle less than three times per week. In the crude model bicycling was only marginally associated with higher values of the carotid distensibility coefficient (p=0.06). Adjusting for objectively measured moderate-and-vigorous physical activity did not affect the association between bicycling and the carotid compliance coefficient, however estimates in the analysis of Young's elastic modules decreased by ~0.10 SD and the association with the distensibility coefficient became stronger and significant (Table 1, model 2). No associations between bicycling and measures of carotid stiffness were observed in girls (Table 1).

Inclusion of the remaining covariates did not change this materially (model 3). However, the difference in carotid distensibility coefficient became borderline significant (p=0.08). Waist circumference and mean arterial pressure (both independently associated with both exposure and outcome) were entered separately into model 3 (Table 2, model 4 and 5). The inclusion did not materially affect the association using the compliance or distensibility coefficient as outcomes. When including Young's Elastic Modulus as outcome, the associations were slightly attenuated.

When analyzing the difference in carotid arterial systolic diastolic diameter and Brachial pulse pressure across the categories of bicycling, boys using the bicycle nearly every day or every day had a --5.0 mmHg (95% CI: -9.2 to -0.85) and -4.6 mmHg (95% CI: -8.0 to -1.18) lower pulse pressure, respectively, compared to boys using the bicycle less than three times per week (adjusted for biological maturity, body height and objectively measured moderate-and-vigorous PA). There was no association

of frequency of bicycle use and larger diastolic arterial diameters in boys (supplement 1). No differences between pulse pressures or diastolic arterial diameter across groups of bicycling were observed in girls (Supplement 1).

Table 2 Associations between the frequency of bicycle usage and measures of carotid arterial stiffness

		< 3 times/week	Nearly every day		Every day		
Model			**Beta (95% CI)**	**P**	**Beta (95% CI)**	**P**	**P for trend**
			Young's elastic modules				
Boys							
	1	*Reference*	-0.34 (-0.77 to 0.11)	0.14	-0.40 (-0.74 to -0.06)	0.02	0.03
	2	*Reference*	-0.37 (-0.82 to 0.08)	0.11	-0.50 (-0.86 to -0.13)	0.01	0.02
	3	*Reference*	-0.32 (-0-81 to 0.18)	0.20	-0.48 (-0.91 to -0.06)	0.03	0.03
	4	*Reference*	-0.25 (-0.74 to 0.25)	0.32	-0.39 (-0.82 to 0.04)	0.07	0.08
	5	*Reference*	-0.38 (-0.88 to 0.11)	0.12	-0.55 (-0.97 to -0.14)	0.01	0.04
Girls							
	1	*Reference*	-0.07 (-0.45 to 0.33)	0.73	0.09 (-0.23 to 0.42)	0.58	0.46
	2	*Reference*	-0.07 (-0.49 to 0.35)	0.73	0.15 (-0.20 to 0.49)	0.41	0.30
	3	*Reference*	-0.19 (-0.65 to 0.27)	0.41	0.07 (-0.32 to 0.47)	0.72	0.50
	4	*Reference*	-0.04 (-0.47 to 0.38)	0.83	0.09 (-0.27 to 0.46)	0.61	0.50
	5	*Reference*	-0.13 (-0.57 to 0.31)	0.56	0.06 (-0.32 to 0.44)	0.77	0.57
			Carotid distensibility coefficient				
Boys							
	1	*Reference*	0.22 (-0.32 to 0.67)	0.34	0.34 (-0.01 to 0.68)	0.06	0.06
	2	*Reference*	0.24 (-0.22 to 0.69)	0.31	0.40 (0.02 to 0.77)	0.04	0.03
	3	*Reference*	0.21 (-0.28 to 0.71)	0.40	0.38 (-0.04 to 0.81)	0.08	0.08
	4	*Reference*	0.15 (-0.35 to 0.75)	0.57	0.30 (-0.14 to 0.73)	0.17	0.12
	5	*Reference*	0.25 (-0.25 to 0.75)	0.32	0.43 (-0.001 to 0.85)	0.05	0.05
Girls							
	1	*Reference*	-0.05 (-0.46 to 0.37)	0.82	-0.27 (-0.61 to 0.08)	0.13	0.10
	2	*Reference*	-0.04 (-0.46 to 0.37)	0.83	-0.27 (-0.61 to 0.08)	0.13	0.10
	3	*Reference*	0.07 (-0.39 to 0.52)	0.78	-0.20 (-0.60 to 0.19)	0.31	0.20
	4	*Reference*	0.001 (-0.45 to 0.47)	0.98	-0.24 (-0.63 to 0.15)	0.23	0.15
	5	*Reference*	0.07 (-0.40 to 0.54)	0.77	-0.21 (-0.61 to 0.18)	0.29	0.17
			Carotid compliance coefficient				
Boys							
	1	*Reference*	0.32 (-0.10 to 0.76)	0.17	0.44 (0.11 to 0.79)	0.01	0.01
	2	*Reference*	0.32 (-0.13 to 0.76)	0.16	0.44 (0.08 to 0.81)	0.02	0.01
	3	*Reference*	0.31 (-0.17 to 0.78)	0.21	0.47 (0.07 to 0.87)	0.02	0.02
	4	*Reference*	0.33 (-0.15 to 0.80)	0.18	0.50 (0.09 to 0.92)	0.02	0.02
	5	*Reference*	0.33 (-0.15 to 0.81)	0.21	0.47 (0.06 to 0.87)	0.02	0.02
Girls							
	1	*Reference*	-0.18 (-0.58 to 0.23)	0.39	-0.20 (-0.54 to 0.13)	0.23	0.25
	2	*Reference*	-0.14 (-0.56 to 0.27)	0.52	-0.18 (-0.52 to 0.17)	0.29	0.34
	3	*Reference*	-0.16 (-0.59 to 0.27)	0.47	-0.27 (-0.65 to 0.10)	0.15	0.15
	4	*Reference*	-0.16 (-0.61 to 0.29)	0.49	-0.25 (-0.63 to 0.14)	0.21	0.21
	5	*Reference*	-0.14 (-0.57 to 0.30)	0.54	-0.27 (-0.65 to 0.11)	0.16	0.15
			Carotid Intima Media thickness				
Boys							
	1	*Reference*	-0.05 (-0.48 to 0.39)	0.83	0.17 (-0.17 to 0.51)	0.34	0.28
	2	*Reference*	0.01 (-0.44 to 0.46)	0.96	0.28 (-0.08 to 0.65)	0.13	0.10
	3	*Reference*	0.03 (-0.46 to 0.51)	0.92	0.32 (-0.10 to 0.73)	0.13	0.11

	4	*Reference*	0.06 (-0.43 to 0.56)	0.80	0.37 (-0.05 to 0.79)	0.09	0.07
	5	*Reference*	0.001 (-0.49 to 0.51)	0.97	0.33 (-0.11 to 0.74)	0.16	0.12
Girls							
	1	*Reference*	0.23 (-0.18 to 0.64)	0.27	0.35 (0.009 to 0.69)	0.04	0.05
	2	*Reference*	0.13 (-0.28 to 0.55)	0.55	0.33 (-0.02 to 0.69)	0.06	0.05
	3	*Reference*	0.01 (-0.44 to 0.46)	0.95	0.32 (-0.10 to 0.73)	0.13	0.10
	4	*Reference*	0.01 (-0.44 to 0.47)	0.96	0.27 (-0.12 to 0.66)	0.17	0.11
	5	*Reference*	0.04 (-0.41 to 0.40)	0.85	0.20 (-0.11 to 0.68)	0.16	0.10

Estimates are standardized values (95% confidence intervals (CI))
Model 1 is adjusted for biological development and body height
Model 2 is adjusted is further adjusted for moderate-and-vigorous physical activity
Model 3 is additionally adjusted for current smoking status, the intake of fruit, vegetable and soft drink consumption, parental educational level, TV-viewing and family history of cardiovascular disease
Model 4 is model 3 additionally adjusted for waist circumference
Model 5 is model 3 additionally adjusted for mean arterial pressure

Associations between commuter mode to school and carotid arterial stiffness

The associations between commuter mode and arterial stiffness are shown in Table 3. As for overall frequency of bicycling, no associations were observed among girls. Boys using their bicycle as mean of commuting had higher carotid compliance and lower values of Young's Elastic Modules compared to passive commuters. However, effect sizes for the distensibility coefficient only reached borderline significance (p=0.08). Boys walking to school had a tendency towards a better buffering capacity and lower arterial wall stiffness compared to passive commuters. Including the remaining confounders from model 3 in the main analysis (excluding moderate-vigorous physical activity) did attenuate the estimates slightly in boys. The effect sizes for bicycle vs. passive commuters were -0.50 (95% CI -1.14 to 0.14), 0.50 (95% CI -0.10 to 1.12) and 0.39 (95% CI -0.25 to 1.03) for Young's elastic modules, compliance and distensibility coefficient, respectively. No differences between the crude and fully adjusted model were observed in girls (data not shown).

Table 3 Associations between commuter mode and measures of carotid arterial stiffness

	Passive	Walking		Bicycling		
Model		**Beta (95% CI)**	**P**	**Beta (95% CI)**	**P**	**P for trend**
Boys (N=172)	*(N=16)*	*(N=44)*		*(N=112)*		
Young's elastic modules	*Reference*	-0.45 (-1.03 to 0.12)	0.12	-0.54 (-1.07 to -0.02)	0.045	0.07
Carotid distensibility coefficient	*Reference*	0.43 (-0.14 to 1.01)	0.14	0.47 (-0.06 to 0.99)	0.08	0.15
Carotid compliance coefficient	*Reference*	0.44 (-0.12 to 0.99)	0.12	0.59 (0.08 to 1.01)	0.02	0.03
Carotid Intima Media Thickness	*Reference*	-0.48 (-1.04 to 0.07)	0.09	-0.18 (0.69 to 0.33)	0.48	0.73
Girls (N=211)	*(N=20)*	*(N=48)*		*(N=143)*		
Young's elastic modules	*Reference*	0.04 (-0.50 to 0.57)	0.89	-0.01 (-0.48 to 0.47)	0.97	0.87
Carotid distensibility coefficient	*Reference*	0.08 (-0.45 to 0.61)	0.76	0.06 (-0.41 to 0.54)	0.79	0.87
Carotid compliance coefficient	*Reference*	0.10 (-0.42 to 0.63)	0.69	0.14 (-0.34 to 0.61)	0.57	0.60
Carotid Intima Media Thickness	*Reference*	0.11 (-0.42 to 0.65)	0.67	0.34 (-0.14 to 0.82)	0.16	0.08

Estimates are standardized values (95% confidence intervals)
The models are adjusted for body height and biological development

Discussion

The main finding of this study was that boys using their bicycle every day of the week displayed a better buffering capacity, lower intrinsic arterial stiffness and, to a lower degree, arterial elasticity compared to those using their bicycle less than three times per week. These associations were independent of important socio-demographic confounders and distending blood pressure. We did not observe any differences in buffering capacity, arterial elasticity or intrinsic wall stiffness across categories of bicycling in girls. Secondly, we observed that boys bicycling to school had a better buffering capacity and lower intrinsic arterial wall stiffness compared to passive commuters, although slightly attenuated in the fully adjusted model.

Our findings support the beneficial effects of PA on arterial stiffness (Nettlefold, McKay, Naylor, Bredin, & Warburton, 2012; Ried-Larsen, 2013; Sakuragi, 2009; Schack-Nielsen, 2005) and we have extended the data by separating the effects of bicycling from other moderate-and-vigorous physical activities. Several observational studies suggest that commuter bicycling is associated with lower levels of metabolic CVD risk factors in children and adolescents (Andersen, 2011a; Cooper, 2006; Cooper, 2008; Ostergaard, 2012). However, these studies have not been able to separate the effects attributable to bicycling from other PA modes that could potentially affect CVD risk. As the Actigraph activity monitors (using the vertical axis) almost does not register moderate-and-vigorous physical during cycling, effect estimates from previous studies linking accelerometer-based PA to metabolic CVD risk factors could be grossly underestimated in countries with a high proportion of bicycling. In this study, objectively measured PA derived from the vertical axis acted as a negative confounder in the sample of boys.

Only a few randomized controlled trials have shown beneficial effects on metabolic CVD risk factor levels of bicycling in adults (Hendriksen, Zuiderveld, Kemper, & Bezemer, 2000; Moller, Ostergaard, Gade, Nielsen, & Andersen, 2011; Oja, 1991), we are only aware of one study assessing the effects of cycling exercise on carotid compliance. Thijssen et al. did not observe an effect of an 8-week cycling intervention on carotid compliance (Thijssen, de Groot, Smits, & Hopman, 2007). However, the study was conducted in an elderly sample (~70 years) and consisted of only three training sessions per week. This is equivalent to the low frequency usage quartile in our study and possibly explaining the discrepancies to our observations.

To the best of our knowledge, only two experimental trials promoting bicycling to decrease metabolic CVD risk factor levels in childhood have been conducted but neither assessed the effect on vascular arterial outcomes. In a smaller randomized controlled trial, we observed an improvement in a CVD risk factor profile among children (n=43) after an eight week intervention promoting commuter bicycling (Ostergaard, 2012). In another trial (n=53), Børrestad et al. observed improvements in cardio respiratory fitness in children who started bicycling to school (Borrestad, Ostergaard, Andersen, & Bere, 2012). This is in line with our observations, that bicycling to school was associated with a better buffering capacity. Thus, promotion of both the total bicycling frequency and commuter bicycling could carry important health benefits. However, more experimental studies are needed to elucidate the effects of bicycling on arterial health in childhood.

As we did not observe any markedly attenuation by adjustment for mean arterial pressure or adiposity it is likely the mechanism responsible for the associations could relate to differences in hemodynamics across the groups of bicyclists. Green et al. observed an increase in blood flow and changed flow pattern in the upper limbs during lower limb cycling at higher, but not lower intensities

(Green, 2002; Green, 2005). This is supported by Thijssen et al. who observed a difference in the haemodynamic response between rhythmic (cycling and walking) and bilateral knee kicking (Thijssen, 2009). The observations suggest that lower limb cycling movements at higher intensities, such as bicycling, exerts a global effect on the hemodynamic patterns. Even though this linkage has not been established for the carotid artery, it is likely that the global effect could induce changes in arterial stiffness in this region of the arterial tree. Repeated changes in flow pattern and shear stress could decrease vasoconstrictor tone through reductions in resting sympathetic outflow, thus increasing the buffering capacity (Green, Spence, Halliwill, Cable, & Thijssen, 2011). In contrast repeated cycles of exercise may increase vascular tone and induce arterial remodeling (Green, 2011). Both theories could explain our observations, if the increase in exercise induced vascular tone is transient.

As in a previous study we observed modification by sex in the associations between moderate-and-vigorous PA and arterial stiffness (Ried-Larsen, 2013). Data in adults suggests that high intensity exercise is required in order to improve arterial function or decrease arterial stiffness (van de Laar, 2010; Green, Maiorana, O'Driscoll, & Taylor, 2004). Thus, a possible explanation could be sex specific preferences in bicycling intensity (or energy expenditure) with boys exerting a higher intensity during bicycling than girls. Unfortunately we do not have data to explore this possibility. Previous studies have reported inconsistency in sex specific cycling intensities (Ostergaard, 2012; Hendriksen, 2000; Oja, 1991) and it is therefore possible that the intensity during bicycling for boys is higher than for girls, and these differences could explain the modification by sex in our sample. An alternative explanation relates to menstrual phase. Hayashi et al. observed a 25% variability of carotid arterial compliance across the menstrual cycle in ten healthy women (Hayashi, 2006). Thus, our observations may have been confounded by lack of control of the phase in the menstrual cycle as there is some

evidence to support that a high PA level is associated with lower levels of estrogen in the luteal phase (De Souza, 1998; De Souza, 2010). Unfortunately, we did not obtain information on menstrual cycle. An alternative explanation includes sex differences in volume of bicycling. As we only obtained measures on frequency of bicycle use we cannot test this alternative hypothesis. However, we did include a question of the time spent on active commuting including the four levels 1 "<5 min", 2 "5-15 min", 3"15-30 min" and 4">30 min" (the latter two were collapsed into one category due few respondents in category 4 (N=2). In a sex stratified post hoc analysis we did not observe a modification of the association between arterial stiffness and commuter bicycling by the duration of commuter bicycling moderated (data not shown). This indicates, at least for commuter bicycling, that the time spend per trip does not influence the estimate. The majority of respondents reporting bicycling every day, also used their bike as a mean of commuting (N_{boys}=65%/N_{girls}=51%, $p<0.001$). It could be speculated that the total volume, expressed by number of trips/week rather than duration/trip could explain our observations. However, the analysis should be interpreted with caution due to low statistical power and the risk of information bias.

Strengths of this study include objectively measured PA and the age homogeneous sample. Although, we are confident that the observed difference between the groups of bicycling usage are independent of moderate-and-vigorous PA it is possible that differences in light intensity PA, overall sedentary behavior, and other PA not registered by the accelerometer, could have confounded our observations as these modes have been associated with adverse metabolic profiles (Atkin, 2013). However, previous observations from the Amsterdam Growth and Health Longitudinal Study only noted an association between vigorous, and not light, PA and Carotid arterial stiffness (van de Laar, 2010). Similarly, as our observations were independent of TV-viewing time and since we did not observe any differences in objectively measured sedentary time (<17 count/10 sec.) or light PA ([17-

499 counts/sec]) across groups of bicycle usage (data not shown), we do not believe that confounding by these parameters could explain our observations.

There are some limitations to the study. First, the usage of bicycling was assessed using self-report and is thus prone to recall bias. However, as the participants were not aware of their arterial properties, the biases are probably non-differential. Second, the large drop-out could induce a selection bias. The drop-outs from childhood to adolescence did not differ from the participants of the study sample in their baseline reporting of commuter bicycling (overall bicycling was not assessed at baseline), BMI or parental educational status six years earlier. Finally, in a post hoc sensitivity analysis, the estimates were weighed according to the probability of participating at follow-up. The estimates were weighted according to overweight, parental educational status and smoking status at baseline (using the *pweight* option in Stata). However, this procedure did not affect the effect estimates. Taken together, we do not suspect that selection bias explains our observations. Third, we used brachial BP for calculation of PP which may overestimate pulse-pressure in the central arteries, especially in young people (Boutouyrie, 1999). This may therefore overestimate our measure of arterial stiffness. This bias would be random as the cohort was homogeneous according to age. Brachial artery pulse-pressure amplifications may, however, differ across gender and CVD risk (Agnoletti, 2012; Regnault, 2012) and could potentially introduce a differential bias if the size of the amplification is related to bicycling and the confounding factors in this sample. Fourth, we do not have information of the duration of or intensity during and volume of bicycling, thus it is unclear to which extent the differences between the groups of bicycling is explained by differences in intensity and/or overall energy expenditure during bicycling. Fifth, as the assessment of the dietary intake was measured crudely this could potentially confound our observations. Sixth, the analysis of commuter mode was only adjusted for body height and biological maturation due to power concerns. Therefore, the associations could be confounded and

the observations should be interpreted with caution. Lastly, due to the cross-sectional design, we cannot exclude the possibility of unknown and residual confounding or infer causality.

Perspectives

The observations from our study may have important preventive and clinical implications. As a frequent use of the bicycle was associated with lower carotid arterial stiffness in boys independent of moderate-and-vigorous PA this suggests, at least in boys, that bicycling could be important to facilitate in order to prevent CVD later in life. As we furthermore observed that the boys bicycling to school had a better buffering capacity, promoting commuter bicycling in childhood could carry important health benefits.

Though the association was not apparent in girls, previous research indicates that bicycling is as effective for decreasing metabolic CVD risk factors levels in girls as well as in boys (Andersen, 2011a; Cooper, 2006; Ostergaard, 2012). It is therefore reasonable to promote bicycling in both sexes. More accurate information on bicycle use (including distribution, volume and intensity) is needed to explore whether the sex difference could be ascribed differences in use.

Strategies promoting total bicycling as commuter bicycling could prove important to decrease CVD risk later in life. However, these strategies should be policy founded as safety and supportive infrastructure is pivotal in succeeding. Further evidence from prospective observational studies and randomized controlled trials are needed to inform policy makers about the needed volume of and intensities while bicycling and the environmental circumstances were bicycle promotion is effective.

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