Retinal vascular diameters in relation to physical activity in Danish children - The CHAMPS Eye Study

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Abstract:

Our objective was to determine associations between retinal vascular caliber and physical activity (PA) in a school-based child cohort. In a prospective study we created a childhood cumulative average PA-index using objectively measured PA (accelerometry) assessed at four periods between 2009 and 2015. Cumulative exposure to PA intensities was estimated. Cross-sectional examinations on biomarkers, anthropometry and ophthalmological data including retinal fundus photographs were performed in 2015. Semi-automated measurements of retinal vascular diameters were performed and summarized into central retinal arteriolar and venular equivalents (CRAE, CRVE). We included 307 participants. Mean age in 2015 was 15.4 years (0.7). The mean CRAE and CRVE were 156.5 µm (2.8) and 217.6 µm (7.7), respectively. After adjusting for age, gender and axial length, more time in PA was independently related to thinner retinal venules (β-coefficient = -1,25 µm/%, 95% confidence interval = -2.20,-0.30, $p<0.01$). Sedentary time was associated with wider venules ($p<0.01$). Furthermore, birthweight (β-coefficient = 0.56 µm/%, 95% confidence interval = 0.18,0.95, $p<0.01$) was associated with CRVE. Blood pressure was associated with thinner retinal arterioles (β-coefficient = -0.19 µm /mmHg, 95% confidence interval = -0.36,-0.01, $p=0.04$). We concluded that children with higher PA in childhood had thinner retinal venular caliber. Our results suggest that PA during childhood positively impacts the retinal microcirculation and that retinal vascular analysis may be a possible assessment to detect microvascular impairments in children with an increased risk of future cardiovascular disease.

Keywords:

Physical activity, Retinal vascular caliber, Microcirculation, Cardiovascular disease prevention, Children, Epidemiology, Sedentary behavior

INTRODUCTION

An increasingly physical inactive lifestyle in the Western World has led to a high number of non-communicable diseases [1,2]. The consequences of modern world lifestyle are already present in childhood with an increased prevalence of overweight, obesity [3] and metabolic syndrome [4]. Concurrent with increasing obesity, blood pressure levels has significantly increased among children and adolescents [5]. High blood pressure is associated with an increased risk of cardiovascular disease [6] and atherosclerosis [7] later in life. Sedentary behavior and physical activity (PA) is associated with both macrovascular [8,9] and microvascular changes in adults [10].

Fundus photography is a non-invasive examination of the retinal microcirculation [11]. Computer-based semi-automated analysis of the retinal vascular diameters allows precise measurements by a thoroughly validated method [11,12].

In adults, retinal vascular changes can predict an increased risk of hypertension, cardiovascular disease [13] and diabetes mellitus [14]. Furthermore, studies have demonstrated retinal vascular changes in children comparable to the changes in adults [15], including less favorable retinal vascular health of thinner retinal arteries and wider venules in children with high blood pressure [15,16]. Thus it appears reasonable to suggest that changes in the retinal microvasculature in children may have the potential to serve as an early marker for endothelial function and subclinical disease [17].

Several methods have been used to quantify PA with the majority based on questionnaires. However, objective measurements with accelerometers provide advantages over subjective self-reports [18].

In cross-sectional studies, objectively measured PA has unambiguously been associated with blood pressure, insulin resistance, lipid levels and other cardio-metabolic biomarkers [19] which are hypothesized risk factors for subclinical and clinical disease manifestations. Only few studies have investigated the association between PA and sedentary behavior and the retinal microvasculature in children [15,20-23] and to the best of our knowledge no studies have used objective data on PA or included several assessments of PA during childhood.

There is a lack of knowledge concerning the link between PA and retinal vascular diameters in children. Furthermore, little is known of the effects of obesity, blood pressure and cardiovascular risk factors on the retinal microvasculature in children. Hence, we conducted the present study with the objective to investigate the association between objective measurements of cumulative PA exposure during childhood and retinal vasculature in a cohort of Danish children and adolescents. We present six years of longitudinal objectively measured data on PA using accelerometry presented as the cumulated exposure through childhood and absolute measures of retinal vascular diameters.

MATERIALS AND METHODS

Study design and participants

The CHAMPS Eye Study was conducted in March to May 2015. The study is based on six years longitudinally objectively measured PA along with cross sectional ophthalmological data. The CHAMPS Eye Study protocol has been described in details previously [24].

The study is a part of The Childhood Health, Activity and Motor Performance School Study Denmark (CHAMPS study-DK); a school-based prospective cohort study of Danish schoolchildren. The CHAMPS Study has been performed in three steps; CHAMPS I (2008-2012), CHAMPS II (2012-2014) and CHAMPS III including the CHAMPS Eye Study in 2015. The CHAMPS study-DK is elsewhere [25].

Participants enrolled in the CHAMPS III seven-year-follow-up (2015) were eligible for the CHAMPS Eye study. In total 840 were invited to participate in the CHAMPS III. In all, 564 responded to the study invitation (339 accepted and 225 declined) which included multiple tests, one of which was the eye examination. The other examinations included: questionnaires, objective tests, physical tests (strength, balance), cognitive tests, Tanner assessment, DXA scans and blood samples.

A total of 307 were included in the CHAMPS Eye Study.

Ethics

The study was approved by the Regional Scientific Ethical Committee of Southern Denmark (ID S-20080047 and S-20140105) and the Danish Data Protection Agency. It was conducted in accordance with the Declaration of Helsinki and in accordance with good epidemiological and clinical practice. All children and parents received detailed information about the study through their school and meetings as well as written information before signed informed consent was given.

Assessment of Physical and Sedentary Activity by Accelerometer Measurements

PA was objectively measured by GT3X+ accelerometers (ActiGraph, Pensacola, Florida, USA)[25] at four different events in 2009, 2010, 2012 and 2015 using identical distribution

protocols. The accelerometer is a hip-mounted, light, solid-state triaxial device, designed to monitor PA in free-living populations. The method has been validated in previous studies and is currently the de facto standard of PA assessment in epidemiological studies in children and adolescents [26]. The accelerometer provides a valid estimate compared to the gold-standard method of assessing PA energy expenditure and is superior to subjective methods [27].

Accelerometer data was converted into information regarding the amount and intensity of PA during the day and subsequently averaged. In accordance with other studies, we defined four intensity domains using accelerometer output ("counts per minute") as sedentary- (SED), light- (L), moderate-to-vigorous- (MV) and vigorous- (V) PA [26]. Average time in the intensity domain was expressed as percentage of total time. The intensity classification algorithm is based on cutoff-points showing excellent intensity classification sensitivity and specificity in accordance with directly measured oxygen uptake in external validation studies [26].

To create an index of the cumulative average PA exposure during childhood we averaged the summary measures from each time-point. Data output from the accelerometers were analyzed using Propero software (version 1.7.4, University of Southern Denmark, Odense, Denmark). The accelerometers were programmed to register PA data every two seconds and subsequently collapsed into a 10 second epoch for analysis according to previous studies [28]. Accelerometers were programmed to start recording the day after distribution. We included data from 06:00 to 23:00 for at least seven full consecutive days. Thereby we potentially included all weekdays and a full weekend. At each PA measurement event data was considered valid if participants had a minimum of three consecutive days, with at least

ten hours per day. Participants were included in analysis of cumulative average PA exposure if they provided valid data for at least two of the potential measurement event.

Ophthalmologic data

Eye examinations were conducted from March to May 2015 at the Department of Ophthalmology, Odense University Hospital, Denmark. We measured cycloplegic (1% Tropicamide) refractive error by a stationary autorefractor (Tonoref II, Nidek, Tokyo, Japan) and calculated the spherical equivalent refractive error. By optical biometry (Lenstar LS 900, Haag Streit AG, Koeniz, Switzerland) we measured the ocular axial length. The eye examination was performed by a trained medical doctor and included fundus photography (Topcon 3D OCT, Tokyo, Japan), biomicroscopy and indirect ophthalmoscopy (Haag-Streit, Köniz, Switzerland).

Retinal Vascular Analysis

We used optic disc centered retinal fundus photographs and the semi-automated computer program IVAN [12] to calculate the diameters of retinal arterioles and venules (Figure 1). The grader manually distinguished arteries from veins and graded the vessels according to the manufacturer's instructions. To provide data on summarized diameters the arterioles and venules were combined according to the "Big 6" method[12], using only the six largest vessels. These were summarized into the central retinal arteriolar and venular equivalent (CRAE and CRVE) and the CRAE-CRVE ratio (AVR). All measurements were performed by one certified grader masked to participant identity and characteristics. The grader reliability has previously been tested and showed high

concordance correlation coefficients [29]. In addition, repeatability of the retinal vascular analyzes of the present study was performed.

Questionnaire

Background information was obtained by questionnaires at baseline (2015)[25]. The socioeconomic status was based on the educational attainment of the mother/female guardian and classified according to a Danish adaptation of the International Standard Classification of Education (ISCED-11). The ISCED scale was combined into two education levels: 1: lower secondary school 2: upper secondary and higher.

Anthropometric data

Body weight was measured to the nearest 0.1 kg on an electronic scale (Tanita BWB-800, Tokyo, Japan) and height was measured to the nearest 0.1 cm using a Harpenden stadiometer (West Sussex, United Kingdom).

Blood pressure

Blood pressure was recorded with a suitable cuff size on the left arm using an automated blood pressure monitor (Welch Allyn®, New York, USA) after resting in sitting position for 5 minutes. Five subsequent values were recorded and the mean of the last three recordings was used in the analysis.

Pubertal self-assessment

The participants were presented with standard pictures showing the pubertal Tanner staging [30] and asked to indicate the stage best referred to their own pubertal stage. Breast

development in girls and pubic hair in boys were used. No participants were in Tanner stage 1. Therefore, the categories were collapsed into the two stages pubertal (Tanner stage 2-4) and fully developed (Tanner stage 5) as in previous studies [31]. For further information and regarding Tanner pubertal self-assessment, please see the CHAMPS Study protocol [25].

Dual-energy X-ray absorptiometry

Fat mass as % of total body mass was measured by dual-energy X-ray absorptiometry (DXA) on GE Lunar Prodigy DXA-scanner (Medical Systems, Madison, Wisconsin, USA) with ENCORE software (version 15) at Hans Christian Andersen Children's Hospital, Odense University Hospital, Denmark. Daily tests for reproducibility were conducted during the study according to standardized procedures.

Statistical Analysis

Results with p-values <0.05 were considered significant. Descriptive statistics were presented as means (standard deviation), and as medians with range for non-parametric data. Skewness and kurtosis test for normality, Shapiro-Wilk Test and q-q plots were used to check assumptions of normality. Residuals plots were inspected to check for the homogeneity of variance assumption.

For continuous variables, the differences between genders were evaluated using the two-sample Student's t-test. For categorical data we used Chi-squared test (χ^2).

Participant maturity and mother's education were examined as dichotomous variables.

Tanner data was used both as categorical and continuous variables showing no difference in the results. Only analyses with the continuous variable were presented.

CRAE and CRVE were analyzed as continuous variables. Intraclass correlation coefficients were performed to assess the repeatability of retinal vascular diameters.

We made multiple linear regression analysis of the retinal vascular diameter in relation to anthropometric data and biomarkers. Associations between the cumulative average time spent in SED and each of the PA intensity levels (LPA, MVPA, and VPA) with CRAE, CRVE and AVR were examined using unstandardized β-coefficients (with 95% confidence intervals) from multiple linear regression models.

The retinal vascular diameters were the dependent variables and PA and other measures were the independent variables. We tested each activity level (SED, LPA, MVPA, and VPA) as both continuous (% PA per day) and categorical (quartiles) variables. Adjusted means were compared across levels of SED and PA domains.

To examine the influence of putative confounders (anthropometry, blood pressure, DXA scan and eye data, all from 2015) we included these variables in sequential models. Three models were constructed to compare the vascular diameters. See table 4.

STATA 14 for Windows (StataCorp LP, College Station, USA) was used for all analyzes.

RESULTS

Characteristics of the included participants from the clinical examination are shown in Table 1. The mean age was 15.4 years (0.7) and 52.4% were boys. Boys were taller and had higher body weight ($p<0.0001$). There was no difference between genders in maturity assessment ($p=0.06$) (Table 1). The mean total body fat mass was 13.7% (6.8) for boys and 27.4% (6.7) for girls ($p<0.0001$), while there was no significant difference of BMI ($p=0.14$) or waist circumference ($p=0.42$) between genders.

The mean axial length was 23.5 mm (0.9) and was statistically significantly longer in boys ($p<0.0001$). Retinal vascular analysis was gradable in all 307 participants (Figure 1). The mean CRAE and CRVE were 156.5 µm (12.8) and 217.6 µm (17.7), respectively. There was no gender difference in CRAE, while the CRVE was thinner in boys ($p<0.01$). To ensure the validity of the retinal vascular measurements, we re-measured a subsample of 10%. The intraclass correlation coefficient was 0.97 (95% confidence interval = 0.94,0.99) for the CRAE and 0.98 (95% confidence interval = 0.97,0.99) for the CRVE.

For 259 participants (84.4%) we had a minimum of two PA measurements, which represent the analytical sample for this exposure. Boys were more active than girls ($p=0.02$ and $p<0.0001$ for LPA and MVPA, respectively) (Table 1).

The follow-up rate for the CHAMPS study was 40.3% over seven years. To address this, comparisons between included and non-included participants were made (Table 2) and showed no statistical significant differences between included and non-included participants, except in age.

Results from multiple linear regression analysis of the association between retinal vascular structure and selected clinical variables are shown in Table 3. After adjustments, a higher blood pressure (systolic and diastolic) was associated with thinner CRAE ($p=0.04$). Higher birthweight ($p<0.01$) was associated with thinner CRVE. Body fat mass ($p=0.04$) was associated with a lower AVR, while height ($p=0.01$) and birthweight ($p=0.01$) were associated with higher AVR.

Multiple linear regression analyzes were performed to test for associations between cumulative average PA and retinal vascular diameters (Table 4). More time spent in SED was statistically significantly associated with increased CRVE in all three models. For all PA intensity levels, associations with CRVE were found. In Model 1 we found that a one

percentage-point higher time spent in PA was associated with a retinal venular diameter decrease of -0.72 µm (p=0.01) for LPA, -1.25µm (p<0.01) for MVPA, and -2.29 µm (p<0.01) for VPA. Additional control for body fat mass, blood pressure and maturity in Model 2 attenuated effect-sizes, but MVPA and VPA remained statistically significant. In Model 3, effect-sizes were further attenuated and no associations remained significant (Table 4).

Table 5 shows the marginal means of the retinal vascular diameter for each quartile of MVPA and SED. Increasing quartiles of MVPA were associated with thinner venules (p=0.01) and increasing quartiles of SED were associated with thicker venules (p=0.005) after multiple adjustments. Children in the highest quartile of MVPA had a mean of 6 µm narrower mean CRVE compared to the lowest quartile.

DISCUSSION

In the present study children with a higher cumulated average engagement in PA had a thinner retinal venular diameter. More time spent in SED was independently associated with thicker retinal venules. Time spent at relatively higher intensity levels was, based on effect-sizes, associated with greater benefits but only SED remained associated with venular diameter in fully adjusted models. Contrary to our expectations, total body fat mass, BMI and waist circumference was not independently associated with retinal vascular calibers. This is the first study including repeated objective measurements of PA in youth linked with retinal vascular calibers.

There is a substantial body of evidence that hypertension, cardiovascular diseases and type 2 diabetes are, at least in part, a result of an increasing inactive lifestyle [2]. Lack of PA

is associated with several chronic diseases and higher mortality [32]. In adult population-based studies, retinal vascular calibers have been associated with obesity [33] hypertension [34], stroke [35] and cardiovascular diseases [11]. Thus, some of the reason for these changes is thought to be directly or indirectly caused by lack of daily PA. In a large population-based, cross-sectional study on adults, beneficial associations were found between higher levels of PA and less microvascular changes and abnormalities [10]. To our knowledge, there are a few cross sectional studies that have examined PA in relation to retinal microvasculature in children so far [15,20-23]. However, not by the same study design. Furthermore, no previous studies have used objective data on PA. All studies besides Imhof et al. [21] estimate the time on PA and sedentary using a single questionnaire administered only once. This method has limitations due to the subjective responses [36] leading to overestimation of PA time [37] and may not accurately reflect the biological progression of lifestyle-related diseases.

Previous studies have found that children who spend more time on PA have wider arterioles [20] and that inactivity is associated with wider venular calibers [23]. Keel et al. [22] report that children that spent less time in PA and more time in sedentary behaviors have an increased likelihood of both retinal arteriolar narrowing and wider venules. Imhof et al. [21] found associations between more indoor PA and thinner venules, using parental administered self-reported PA. Our data supports these studies.

The mean CRAE and CRVE of our study were comparable to the results in other studies in children [16,20,38], but thinner than in two other studies [15,21]. We found that girls had wider venules, but found no significant gender difference in arterioles. These findings were also in accordance with the results of others [15,23]. Hanssen et al. found that girls had both wider

arteriolar and venular diameters compared to boys. Finally, some studies did not find a difference [38] and others did not report this [16,20,21].

Besides these findings authors also report associations between increasing obesity, BMI and blood pressure to venular dilatation and arterial narrowing [15,22,23]. Higher BMI was associated with wider retinal venular diameters [15,38]. Higher systolic blood pressure was associated with wider retinal venular diameter [16] and a thinner arteriolar diameter [15,16]. These associations were not found in the present study which was unexpected. In contrast, we found that lower birthweight was associated with wider venular diameters. Furthermore, an increase in blood pressure was associated with thinner arterioles, and the AVR was associated with body fat mass, waist circumference and height. The conflicting results or lack of consistency might be caused by the heterogeneity of the study designs and the participants. However, there seems to be a consistent association between PA and retinal vascular caliber.

The pathophysiological mechanisms underlying our findings of a positive relationship between increased PA and microvascular health are unknown. The retinal vascular changes may mirror several different systemic and pathophysiological processes. Theories for possible biological explanations include:

1 An anti-inflammatory mechanism due to beneficial effects of PA. While exercise possesses anti-inflammatory properties, increased inflammatory markers are associated with wider retinal venules [39].

2 An increase in blood flow due to exercise, which subsequently leads to an increased intravascular pressure and further causes stress on the vessel wall and finally triggers increased nitric oxide production and an endothelium-dependent vasodilatation [40]. In

contrast, decreased PA might lead to increased blood pressure and hypertension, followed by impaired endothelium-dependent vasodilatation.

3 An inactivity-induced decreased blood flow, which might prompt production of reactive oxygen species and other endogenous vasoconstrictors resulting in altered retinal microvasculature [41].

4 An inactivity-associated higher BMI and obesity. While obese individuals have an increased total blood volume [42], the veins are considered the main capacitance of the total body blood volume explaining the venular dilation.

5 An inactivity-induced obesity, which leads to altered microvascular autoregulation due to adipocytokines, which is shown to modulate endovascular nitric oxide synthesis and vasodilatation [43].

The study has a number of strengths. The retinal vascular analysis method and the IVAN program have been validated in several previous studies and showed high repeatability in the present study.

We used longitudinal, objective measurements by accelerometry which was an advantage as accelerometry is considered to provide high validity in epidemiological studies [27]. Limitations with studies on subjective PA data from questionnaires have been discussed in a previous publication [24]**.** However, the accelerometers do have some limitations due to technical specifications, which might have resulted in underestimation of PA [24].

Even thought our study was relatively small it includes a sample of healthy children and adolescents with a narrow age span. The participants were free of the influences from systemic diseases that could influence the retinal vascular analysis.

The availability of only one assessment of retinal calibers prevented us from inferring on the temporal relationship between variables. Therefore, the natural history of the retinal vascular changes and their relationship with PA, anthropometry and biomarker still remains uncertain.

It is a strength of oyr study that we collected blood samples, DXA scans, puberty assessment by Tanner stages and socioeconomic information from our participants. Thereby, we could incorporate this information in our analyses. Finally we cannot exclude the possibility of residual or unmeasured confounding. While we were able to adjust our analyses for several putative confounding variables, including anthropometric, systemic and socioeconomic variables, other confounders might be releveant, including diet quality and quantity, nutrition [44], blood glucose level[45], and the influence of residual confounding cannot be rejected

CONCLUSION AND PERSPECTIVE

Engaging in PA is a valuable and inexpensive method to prevent obesity, diabetes mellitus and cardiovascular diseases later in life [2]. Meanwhile, there is evidence that the PA pattern and cardiovascular disease risk factors can be tracked from as early as childhood and adolescence into adulthood [46]. It is important to better understand the relationship of PA, disease risk factors and vascular diameters, if the root of certain diseases is already laid down in childhood. By being able to identify retinal vessel diameters as a subclinical risk factor marker for increased risk of hypertension and cardiovascular disease later in life, it might be possible at an early stage to identify the children who will benefit from targeted preventive efforts.

Although, we hypothesized that retinal diameters would be associated with PA, this was only statistically significant in CRVE. This could be due to the relatively young participants and caused by a still undamaged retinal arteriolar autoregulation.

In conclusion, we present six years of longitudinal objectively measured PA presented as the cumulated average exposure through childhood. Our results bring a contribution to this field of research, which is mainly represented by subjective data from questionnaires. We report a cross-sectional association between a higher average engagement in PA and thinner retinal venules. Similar changes in retinal vascular diameters are associated with hypertension, obesity and cardiovascular disease in adults.

The significance of the vascular changes in inactive children in the present study calls for further research to study the effect of PA on the retinal vessels.

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Table 1 Selected Characteristics of CHAMPS Eye Study Participants and According to Gender

	All Mean (SD)	**N**	**Boys Mean (SD)**	**Girls Mean (SD)**	**N (boys) (%)**	**P value**
Age (years)	15.4 (0.7)	307	15.4 (0.7)	15.4 (0.7)	161 (52.4)	0.97
Height (cm)	172 (8)	301	176 (8)	168 (6)	160 (53.2)	<0.0001
Weight (kg)	61 (10)	300	63 (11)	58 (8)	160 (53.3)	<0.0001
BMI (kg/m^2)	20.2 (2.5)	300	20.0 (2.5)	20.5 (2.5)	160 (53.3)	0.14
Waist circumference (cm)	69 (7)	301	75 (6)	74 (8)	160 (53.2)	0.42
Birthweight (g)	3472 (676)	273	3571.4 (689.3)	3334.1 (677.8)	145 (53.1)	<0.005
Pubertal assessment***		301			160 (53.2)	
Tanner 1	0		0	0		
Tanner 2	10 (3.3%)		4 (2.5%)	6(4.3%)		
Tanner 3	26 (8.6%)		8 (5%)	18(12.8%)		
Tanner 4	164 (54.4%)		91 (56.8%)	73(51.8%)		
Tanner 5	101 (33. 6%)		57 (35.6%)	44 (31.2%)		0.41
Maturity assessment		301			160 (53.2)	
Pubertal (Tanner stage 2-4)	200 (66.8%)		103 (64.4%)	97 (68.8%)		
Fully developed (Tanner stage 5)	101 (33.2%)		57 (35.6%)	44 (31.2)		0.06
Blood Pressure – systolic (mmHg)	110 (9)	295	112 (9)	108 (7)	157 (53.2)	<0.0001
Blood Pressure – diastolic (mmHg)	63 (6)	295	62 (6)	64 (6)	157 (53.2)	<0.01
DXA total % body fat mass	20.6 (9.7)	255	13.7 (6.8)	27.4 (6.7)	126 (49.4)	<0.0001
Home environmental markers						
Mothers BMI	25.0 (3.7)	230	24.7 (3.8)	25.3 (4.2)	117 (50.9)	0.20
Mothers education		249			125 (50.2)	
Less than tertiary education	110 (44%)		54 (43%)	56 (45%)		
Completed any tertiary education	139 (56%)		71 (57%)	68 (55%)		0.10
Ophthalmological data		307				
Cycloplegic SE (D)	0.5 (1.5)		0.4 (1.4)	0.6 (1.5)	161 (52.4)	0.15
AL (mm)	23.5 (0.9)		23.7 (0.9)	23.1 (0.9)	161 (52.4)	<0.0001

Retinal vascular calibers		307			161 (52.4)	
CRAE (μm)	156.5 (12.8)		155.4 (11.4)	157.7 (14.2)		0.12
CRVE (μm)	217.6 (17.7)		215.1 (16.1)	220.2 (18.0)		0.002
AVR	0.72 (0.06)		0.73 (0.06)	0.72 (0.06)		0.12
Physical Activity (%)*		259			128 (49.4)	
Sedentary activity	69.0 (5.2)		67.6 (5.6)	70.4 (4.4)		<0.0001
Light activity	24.7 (3.6)		24.7 (3.8)	23.6 (3.3)		0.02
Moderate-to-vigorous activity	6.9 (2.2)		7.8 (2.3)	6.0 (1.6)		<0.0001
Vigorous activity	2.8 (1.2)		3.2 (1.3)	2.3 (0.9)		<0.0001

Abbreviations: BMI: Body mass index; DXA: Dual-energy X-ray absorptiometry scan; SE: Spherical equivalent; D: Diopters; AL: Axial length; CRAE: Central retinal arteriolar equivalent; CRVE: Central retinal venular equivalent; AVR: arteriolar to venular diameter ratio.

Data are presented as mean (SD), range or n (%).

*Cumulative average physical activity assessment from 2009-2015.

Table 2 Comparison of Baseline Characteristics between Included and Non-included Participants in the CHAMPS-Eye Study

	CHAMPS Eye Study participants	N	Not included participants	N	P value
Age (years)	8.9 (0.7)	228	9.5 (0.9)	533	<0.001*
Sex (% girls)	53	307	47	533	0.11**
Body mass index (kg/m^2)	16.4 (1.8)	201	17.0 (2.4)	532	0.02
Body fat (%)	19.1 (7.4)	200	21.1 (8.3)	500	0.03
Sexual maturity (% tanner 1)	49	196	50	494	0.83
Systolic blood pressure	101.3 (7.2)	195	103.3 (7.8)	520	0.12
Insulin (IU/l)	3.6 (2.0)	178	4.1 (2.1)	428	0.71
Triglyceride (mg/dl)	55.0 (22.6)	178	58.3 (25.4)	428	0.63
HDL-cholesterol (mg/dl)	64.8 (13.1)	178	65.2 (14.1)	428	0.31
Mothers education (% any tertiary)	45	205	43	437	0.74
Moderate physical activity/day (%)	5.1 (1.4)	206	5.0 (1.4)	471	0.51
Vigorous physical activity/day (%)	2.8 (1.2)	206	2.8 (1.4)	471	0.31

Children attending grades 2-4 in 2008 (eligible for CHAMPS-Eye study) were included in comparisons. Crude descriptive data is presented as mean (SD). N=79 participants from the CHAMPS Eye Study did not participate in the CHAMPS baseline study in 2008.

P-values are from linear regression analysis adjusting for age and sex unless otherwise noted.

*Unpaired t-test.

**Chi-squared test.

Table 3 Multiple Linear Regression Analysis of Retinal Vascular Diameter and AVR in Relation to Anthropometry and Biomarkers

Parameter	N	CRAE			CRVE			AVR		
		β-coefficient	95% CI	P-value	β-coefficient	95% CI	P-value	β-coefficient	95% CI	P-value
Anthropometry										
DXA total % body fat mass (%)	255	-0.15	-0.35,0.05	0.15	0.13	-0.15,0.41	0.37	-0.001	-0.002,-0.00003	0.04*
BMI (kg/m^2)	252	-0.36	-0.93,0.20	0.21	-0.32	-1.10,0.46	0.42	-0.001	-0.003,0.002	0.67
Waist circumference (cm)	253	-0.05	-0.15,0.04	0.29	-0.05	-0.18,0.09	0.50	-0.0001	-0.0006,0.0004	0.75
Height (cm)	258	0.09	-0.12,0.30	0.42	-0.28	-0.57,0.01	0.05	0.001	0.0003,0.0024	0.01*
Birthweight (kg)	250	0.01	-0.2,0.2	0.97	-0.4	-0.1,-0.1	<0.01*	0.001	0.001,0.002	0.01*
Maturity	257	-3.03	-6.34,0.29	0.07	-0.26	-4.85,4.33	0.91	-0.012	-0.03,0.01	0.17
Blood Pressure – systolic (mmHg)	249	-0.19	-0.36,-0.01	0.04*	-0.22	-0.46,0.02	0.07	-0.0001	-0.001,0.001	0.78
Blood Pressure – diastolic (mmHg)	249	-0.24	-0.47,-0.004	0.04*	-0.04	-0.35,0.28	0.82	-0.001	-0.002,0.0003	0.13

Abbreviations: CI: Confidence interval; CRAE: Central retinal arteriolar equivalent; CRVE: Central retinal venular equivalent; AVR: arteriolar to venular ratio.

The β-coefficient describes the change of the retinal calibers in µm per one unit of the parameters.

Adjusted for age, gender and axial length.

*P < 0.05

Table 4 Multiple Linear Regression Analysis of Retinal Vascular Diameter in Relation to Physical Activity

	Model 1 (N = 259)			Model 2 (N = 246)			Model 3 (N = 214)		
Predictor	**β-coefficient**	**95% CI**	**P-value**	**β-coefficient**	**95% CI**	**P-value**	**β-coefficient**	**95% CI**	**P-value**
CRAE									
Sedentary behavior	0.09	-0.20,0.37	0.54	0.16	-0.14,0.46	0.29	0.22	-0.11,0.54	0.19
Light physical activity	-0.07	-0.48,0.33	0.73	-012	-0.54,0.30	0.62	-0.22	-0.68,0.24	0.35
Moderate+vigorous physical activity	-0.33	-1.02,0.37	0.36	-0.62	-1.37,0.12	0.10	-0.63	-1.42,0.16	0.12
Vigorous physical activity	-0.43	-1.66,0.80	0.49	-1.03	-2.37,0.30	0.13	-0.88	-2.30,0.54	0.23
CRVE									
Sedentary behavior	0.56	0.18,0.95	<0.01*	0.44	0.03,0.84	0.03*	0.43	-0.000,0.86	<0.05*
Light physical activity	-0.72	-1.27,-0.16	0.01*	-0.53	-1.10,0.04	0.06	-0.57	-1.18,0.04	0.06
Moderate+vigorous physical activity	-1.25	-2.20,-0.30	<0.01*	-1.02	-2.03,-0.02	<0.05*	-0.86	-1.91,0.19	0.11
Vigorous physical activity	-2.29	-3.96,-0.61	<0.01*	-1.90	-3.70,-0.10	0.04*	-1.38	-3.26,0.51	0.15
AVR									
Sedentary behavior	-0.001	-0.002,0.001	0.05	-0.001	-0.002,0.001	0.37	-0.001	-0.002,0.001	0.64
Light physical activity	0.002	0.0001,0.004	<0.05*	0.001	-0.001,0.003	0.23	0.001	-0.001,0.003	0.45
Moderate+vigorous physical activity	0.002	-0.001,0.006	0.17	0.000	-0.003,0.004	0.78	0.000	-0.003,0.004	0.88
Vigorous physical activity	0.005	-0.001,0.011	0.11	0.001	-0.006,0.008	0.66	0.000	-0.007,0.007	0.98

Abbreviations: CI: Confidence interval; CRAE: Central retinal arteriolar equivalent; CRVE: Central retinal venular equivalent; AVR: arteriolar to venular diameter ratio.

The β-coefficient describes μm per 1 % change in physical activity.

Model 1: adjusted for age; gender and axial length.

Model 2: Model 1 plus adjusted for total % body fat mass, blood pressure (systolic and diastolic) and Tanner stage.

Model 3: Model 2 plus adjusted for birth weight, mother's BMI and mother's education.

*P < 0.05

Table 5 Mean Retinal Arteriolar and Venular Diameter in Relation to Quartiles of Sedentary Activity and Moderate+Vigorous Physical Activity

Parameter	CRAE			CRVE		
	Mean	95% CI	P-value*	Mean	95% CI	P-value*
Sedentary activity						
1st quartile, <65.52	158.2	138.4,180.2		215.4	190.4,251.3	
2nd quartile, 62.52-69.13	157.8	139.3,176.5		216.8	190.1,243.8	
3rd quartile, 69.14-73.01	155.9	136.1,173.5		219.6	191.5,251.4	
4th quartile, >73.01	155.0	132.2,174.1	0.54	219.4	193.7,246.3	0.005
Moderate+vigorous physical activity						
1st quartile, <5.18	157.3	134.9,173.6		221.0	196.4,247.4	
2nd quartile, 5.18-6.49	155.2	136.3,175.6		217.4	191.9,250.9	
3rd quartile, 6.50-8.28	157.4	142.5,176.5		217.0	190.2,245.4	
4th quartile, >8.28	157.0	134.7,179.5	0.36	215.8	191.0,241.9	0.010

Abbreviations: CI: Confidence interval; CRAE: Central retinal arteriolar equivalent; CRVE: Central retinal venular equivalent

Adjusted for age; gender and axial length.

Values for retinal variables are mean diameter (μm) with 95% confidence interval for each quartile. PA variables are % wear-time. N = 259.

*The P-value across all four quartiles from regression analysis.

Figure Legends

FIGURE 1 Retinal vascular analysis in CHAMPS Eye Study Participants

A retinal photography from one CHAMPS Eye Study participant analyzed by the IVAN program. The grid is automatic placed in relation to the optic disc (left). The largest arterioles (red) and venules (blue) passing through Zone B are identified (right).

DD: disc diameter.

FIGURE 1