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Body fat evolution as predictor of retinal microvasculature in children

Carola J.C. Van Aart[1], Nathalie Michels[1], Isabelle Sioen[1,2], Annelies De Decker[1], Tim S. Nawrot[3,4] and Stefaan De Henauw[1,5]

[1]Faculty of Medicine and Health Sciences, Department of Public Health, Ghent University, Ghent, Belgium

[2]Faculty of Bioscience Engineering, Department of Food Safety and Food Quality, Ghent University, Ghent, Belgium

[3]Centre for Environmental Sciences, Hasselt University, Hasselt, Belgium

[4]Department of Public Health and Primary Care, Leuven University, Leuven, Belgium

[5]Department of Health Sciences, Vesalius, University College Ghent, Ghent, Belgium

Corresponding author

Carola J.C. van Aart, MSc, Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185 4K3, 9000 Ghent, Belgium. Phone: 0032 9 332 3685. Fax: 0032 9 332 4994. E-mail: Carola.vanAart@ugent.be

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Abstract

OBJECTIVES: Microvascular changes may represent an underlying mechanism through which overweight contributes to cardiovascular disease development. Therefore, the aim of this study was to investigate whether changes in children's body fat over time are associated with the retinal microvasculature, a marker of cardiovascular aging.

METHODS: In a longitudinal design, 171 healthy Flemish children (53.8% boys) were followed-up for 7 years (2008-2015), aged 2.7-8.1 years at baseline. Z-scores of body mass index (zBMI; 4.1% overweight), waist circumference (zWC) and fat mass index (zFMI by BODPOD) were obtained using standardized protocols during each visit. Retinal arteriolar (CRAE) and venular equivalents (CRVE) were measured from digital retinal photographs (2015) using IVAN software. Cross-sectional and longitudinal associations between changes in body fat and retinal microvasculature were explored using multivariable regression analysis, while controlling for age, sex, mean arterial pressure, alternate retinal caliber, physical activity, diet and birth weight.

RESULTS: In cross-sectional analysis, children with high zFMI had a higher CRVE, but only in boys (β=0.25,p=0.02). In addition, boys with high zFMI had also a lower CRAE to CRVE ratio (β= -0.26,p=0.03). No associations were seen with the CRAE, or between zBMI or zWC and the retinal microvasculature. Only changes in zFMI over time were found to be positively associated with the CRVE in boys (β=0.38,p=0.01).

CONCLUSIONS: Our analysis over a 7-year period shows that changes in body fat during childhood are already associated with the CRVE (especially in boys).

Introduction

Overweight is one of the main 21st century public health challenges. The World Health Organization has estimated that worldwide 1.9 billion adults were overweight in 2014 and 13% of these were obese.[1] Also the number of children with overweight and obesity has increased dramatically since 1980.[2] Overweight and obesity are well-known risk factors for the development of cardiovascular diseases.[3] Adverse effects of overweight on cardiovascular health are already seen in children: children with overweight are more likely to develop cardiovascular diseases in adulthood and even childhood obesity is associated with large vessel atherosclerotic lesions.[4-6] Therefore, identifying early risk factors in the development of cardiovascular diseases during childhood is of great importance.

The retinal microcirculation is an easy accessible non-invasive tool, which can be used as a preliminary marker for cardiovascular disease in later life, even before clinical manifestations occur.[7] In adults, changes in the retinal microcirculation (smaller arteriolar and larger venular caliber) have been linked to hypertension, myocardial infarction and stroke.[8] Body composition seems to induce alterations in the retinal microcirculation. In cross-sectional studies, increasing body mass index (BMI) and body fat percentage in children were associated with smaller arteriolar and/or wider venular caliber.[9-15] Tayler et al. also demonstrated similar cross-sectional relationships between waist circumference and retinal microcirculation in 6-year-olds.[11] To our knowledge only one longitudinal study has been published regarding the relation between body composition and retinal microvasculature in children. Kurniawan et al. studied the relation between changes in BMI and retinal indicators over 5-years in Singaporean children, and found a positive association between children's BMI and venular caliber.[16] However, these findings may not be representative for a Caucasian population, due to ethnic differences in the retinal microcirculation.[17]

Therefore, the aim of this study was to investigate in healthy Flemish children whether changes in body fat over time are associated with the retinal microvasculature. We studied associations over a 7-year timeframe with multiple measurement waves, in which several

markers of adiposity where tested: BMI, fat mass index and waist circumference reflecting abdominal adiposity.

Subjects and methods

Study population

In 2015, 242 Belgian children and adolescents aged 9 to 15 years from the municipality Aalter and surroundings, participated in the sixth wave of a large longitudinal study. The baseline survey was conducted in spring 2008, with follow-up surveys in spring 2010, 2011, 2012, 2013 and 2015, as part of different study projects. The baseline survey in 2008 was part of the IDEFICS study (Identification and prevention of dietary- and lifestyle- induced health effects in children and infants).[18] Children were invited to participate in the second round of the IDEFICS study in 2010, and the baseline survey of the ChiBS study (Children's Composition and Stress) in 2010[19], which were organized at the same time. Follow-up surveys of the ChiBS study were conducted in 2011 and 2012. In 2013 and 2015, the follow-up surveys were part of the Forces study. We included children based on the availability of retinal photographs and blood pressure data in 2015, as can be seen in the flow-chart in Figure 1. After exclusion of children without retinal photographs (N=23), blood pressure data (N=1), physical activity data (N=20) and birth weight (N=27), this resulted in 171 subjects in 2015, of which all were Caucasian, except two from African origin. Body composition parameters and retinal microvasculature did not significantly differ between included and excluded children. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and the project protocol was approved by the Ethics Committee of the Ghent University Hospital. A written informed consent was obtained from the parents and a verbal assent from the children. In 2013 and 2015, children older than 12 years also signed a written informed consent.

Anthropometry

At each survey, children's height (m) and weight (kg) were measured. BMI was calculated for all surveys, by dividing weight with height squared (kg/m^2). The Flemish growth reference data of 2004 were used to compute the standard deviation score of BMI (zBMI), to adjust for age and sex. Children with underweight, normal weight and overweight were defined using cut-off values of Cole et al.[20]

Waist circumference (WC) was also measured at all surveys, except in 2011. It was defined as the mid-point between the top of the iliac crest and the lower coastal border, and measured

using a circumference measuring tape. An age and sex specific waist circumference z-score (zWC) was calculated by subtracting its sub-group mean from the raw score and dividing by the standard deviation. Herein, children were defined with excess abdominal adiposity based on cut-off values proposed by Fredriks et al. (>1.3 standard deviation).[21]

Body composition (i.e. fat mass) was determined in 2011, 2012, 2013 and 2015 using the BODPOD® air-displacement plethysmography device (Life Measurement Inc., Concord, CA, USA; http://www.lifemeasurement.com/). In accordance to the manufacturer's guidelines the BODPOD® was calibrated daily. Bathing suits and swim caps were worn by the children to rule out air trapped in cloth and hair. Thoracic gas volume was predicted by the BODPOD® software with a validated child-specific equation and the formula of Wells et al. was used to calculate the fat mass.[22,23] The fat mass index (FMI) was transformed into a standard deviation score of FMI (zFMI) using British reference data.[24] Finally, children with excess overall adiposity were defined based on the 75th percentile.

Retinal microvasculature

Retinal photographs (Canon 45° 6.3-megapixel digital nonmydriatic retinal camera) were taken from both eyes at the last follow-up survey (2015). The retinal vessel measurement system IVAN (University of Wisconsin, Madison, Wisconsin, USA) was used to measure retinal vessel diameters by one trained grader. On beforehand, a scaling factor was calculated to adjust for magnification differences, which could be introduced by camera optics, patient position or scanner resolution. The distance in pixels from the center of the disc to the center of the macula were measured in a random sample of 40 photographs, and were used for the grid of the disc diameter. All arterioles and venules in an area 0.5 to 1 disc diameter from the optic disc margin were measured and summarized in the central retinal arteriolar and venular equivalent (CRAE and CRVE), using formulas developed by Hubbard et al.[25] and later modified by Knudtson et al.[26] The arteriolar-to-venular ratio (AVR) depicts the ratio between the CRAE and CRVE. Both retinal photographs, if present (N=150), were used to calculate the retinal microvasculature. In case only one photograph was available (N=21), this photograph was used to calculate the aforementioned microvascular indexes.

Cardiovascular parameters

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in 2015 with an automated device (Welch Allyn, USA). The child had to rest for five minutes prior to the test. Blood pressure was initially measured twice, with a two minute resting period in between. If the systolic and diastolic values differed more than 5% between the two measurements, a third measurement was carried out. The average of the two or three measurements was calculated. The mean arterial pressure (MAP) defined as the average pressure throughout the cardiac cycle was calculated using the following formula: 1/3(SBP) + 2/3(DBP).

Covariates

Physical activity was obtained using questionnaires filled in by the parents during last follow-up survey, based on weekly hours in a sports club and playing outside. Dietary habits were acquired using parental food frequency questionnaires, from which the sugar and fat propensity ratio were calculated using the formulas proposed by Lanfer et al.[27] Children's birth weight was obtained via the parental questionnaire. The pubertal status of children was obtained using the tanner stage via questionnaires filled in by the children during the last follow-up survey. The original five tanner categories were recoded into three categories: prepubertal (no signs of puberty, category 1), peripubertal (signs of puberty, categories 2 and 3) and pubertal (categories 4 and 5).

Statistical analyses

We used Spearman correlations coefficients to determine the correlation between the retinal microvasculature (CRAE, CRVE and AVR) and body composition (zBMI, zWC and zFMI), age and blood pressure. Hereafter, linear regression models assessed the relation between body composition and retinal microvasculature. In the first model, cross-sectional (2015) relationships between continuous predictors (zBMI, zWC and zFMI) and CRAE, CRVE and AVR were explored. Testing the effects of CRVE and CRAE independent of each other is important since it might shed light on specific pathophysiological mechanisms (cf. different anatomical structure of arterioles and venules) and since they are major determinants of each other presumably due to their shared genetic and environmental determinants.[28] In contrast to this independent effect, the AVR is reflecting a combination of the two (lower AVR by combination of smaller CRAE and larger CRVE), as an even stronger marker of cardiovascular aging. In a

second model, similar linear regressions were run with the anthropometry parameters as categorical variables (underweight/normal/overweight based on zBMI; normal/excess abdominal adiposity based on zWC; normal/excess adiposity based on zFMI). Finally, the linear regression models were performed with the baseline and continuous change in zBMI, zWC and zFMI between two study surveys as predictor. No significant interactions were identified between zBMI, zWC and zFMI and sex (p between 0.22 and 0.96). Nevertheless, in a less adjusted model a significant interaction was found between zFMI and sex for CRVE (p=0.04) and AVR (p=0.049), therefore on explorative basis stratification on sex took place for zFMI. All regression models were corrected for age, sex, MAP, the alternate retinal vessel caliber (CRVE adjusted for in models with CRVE and vice versa), physical activity, sugar and fat propensity ratio and birth weight, and assumptions of linear regression analysis were met for all models. Additionally, we only adjusted for age, sex, MAP and the alternate retinal vessel caliber (CRVE adjusted for in models with CRVE and vice versa) in supplemental regression analyses to increase the sample size. Socio-economic status was not included as confounder in the regression models, as it was not found to be a significant predictor of the retinal microvasculature. The statistical analyses were performed two-sided with SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA.), considering $p<0.05$ as significant. The Bonferroni post hoc test was additionally used to correct for multiple testing.

Results

Subject characteristics

In 2015, 171 children participated in the sixth follow-up survey. Table 1 shows the characteristics of the study population per study wave. The CRAE was significantly different between boys and girls (p=0.03), but not for the CRVE and AVR. A higher percentage of girls were pubertal compared to boys (20.5% and 16.5% respectively). Significant negative correlations were found between MAP and CRAE (p=0.009) and AVR (p<0.001). In addition, a borderline significant negative correlation was identified between zBMI and AVR. Age, zWC and zFMI were not correlated with the CRAE, CRVE and AVR.

Cross-sectional associations between body composition and retinal microvasculature

Table 2 presents the cross-sectional associations between body composition and CRAE, CRVE and AVR, in which no associations were found. To identify participants with overweight or excess adiposity, participants were categorized in two or three groups according to zBMI, zWC and zFMI scores, as seen in Table 3. Compared to those with low zFMI, children with high zFMI had a higher CRVE but only in boys (β=0.25,p=0.02). In addition, boys with high zFMI had also a lower AVR (β= -0.26,p=0.03). A borderline association was seen between zWC and CRVE (β=0.11,p=0.08). No significant association was seen for zBMI and retinal microvasculature. After applying the Bonferroni correction (p-value threshold 0.017) only the association between zFMI and CRVE remained significant.

Associations between change in body composition and retinal microvasculature

The relation between a continuous change in zBMI, zWC and zFMI over time and CRAE, CRVE and AVR is given in Table 4. Changes in zBMI and zWC were not related with CRAE, CRVE or AVR. A change in zFMI over 4, 3 and 2 years was positively associated with CRVE (β=0.41, β=0.36 and β=0.38 respectively), but only in boys. Finally, all associations between change in zFMI and CRVE remained significant after Bonferroni correction (p-value threshold 0.017).

Similar significant associations, both cross-sectional and over time, on a larger subset of participants were seen between body fat and CRAE, CRVE and AVR while only controlling for

age, sex and MAP, as presented in the Supplementary information. In addition, compared to those with normal weight, children with overweight had a higher CRVE (β=0.13,p=0.02).

Discussion

Our study provides new data on the relationship between body composition and retinal microvasculature in children, a marker for cardiovascular disease in later life. Higher total body fat was associated with retinal venular widening especially in boys. Children with excess adiposity over time had less favourable retinal outcomes, e.g. wider venules. No significant associations were found for the arterioles.

CRVE

Cross-sectional data showed a positive relation between higher body fat and CRVE. This finding is in accordance with previous cross-sectional studies.[9,11,12,14,15] In contrast to a longitudinal study, where an increase in BMI over 5 years was associated with wider venules,[16] we did not find a significant relation between increasing BMI and higher CRVE when using longitudinal body composition data. However, longitudinal information about zFMI was found to be associated with higher CRVE. Surprisingly, associations with overall adiposity were only found for boys in our study. This might be explained on the basis of differences in circulating hormones between boys and girls during puberty, as testosterone plays a major role in boys and estrogen in girls. Circulating testosterone during puberty enhances fat-free mass, while estrogen in girls is thought to enhance fat storage.[29] Despite this, estrogen is also thought to be a potential protective factor for cardiovascular diseases,[30] which might have influenced the association between zFMI and CRVE in girls. However further research is still needed to fully explore these relationships. In contrast to other studies, our results were overall more explicit in the extremes, i.e. in children with excess adiposity, suggesting an exponential relation between body fat and retinal microcirculation.

CRAE

The CRAE did not seem to be associated with body composition. Literature however suggests a negative relation between CRAE and body composition.[13-15] The Dutch Generation R study noticed that children with a higher BMI and body fat mass had smaller arterioles.[13]

AVR

In our study we identified one significant association between body composition and AVR. In cross-sectional analyses we noted that boys with excess overall adiposity had a smaller AVR, which is in accordance with the studies of Siegrist et al. and Hanssen et al. did.[10,15] The first showed that higher BMI was related with smaller AVR,[10] and the second noticed similar associations for BMI, percentage body fat and waist circumference.[15]

Pathophysiological mechanisms

The pathophysiological mechanism underlying the relationship between higher CRVE and excess body fat remains unclear. However, there are several plausible explanations. First, obese individuals have a larger blood volume compared to non-obese individuals, which may widen the retinal venules, as they are known to play a role in regulating blood flow.[31] Secondly, obesity is characterized by a systemic low-grade inflammatory status. Pro-inflammatory proteins from the adipose tissue, can enhance the production of C-reactive protein (CRP) and tumor necrosis factor alpha.[32,33] Gishti et al. already noticed that increased CRP was associated with wider venules in children.[13] Also leptin, a mediator of long-term regulation of energy balance, is thought to play a role. After all, leptin is increased in obese individuals and can alter the nitric oxide synthesis leading to vasodilation.[32,33] The effect of leptin on the retinal microvasculature has been described by Siegrist et al, where higher leptin levels in children were associated with higher CRVE and smaller AVR.[10]

Clinical importance

Retinal blood vessels share developmental and anatomical similarities with the microcirculation of the heart and brain, and are therefore thought to be a good surrogate for the systemic microcirculation.[34] As noted earlier, wider venules and smaller arterioles were associated with occurrence of stroke, hypertension and myocardial infarcts.[8] This stresses the importance of the retinal microvasculature as an early non-invasive marker for cardiovascular diseases. It might also become useful in clinical settings for clinicians to identify patients who are at risk of future cardio- and cerebro-vascular events.[34]

Strength and weaknesses

To our knowledge, this is the first European study investigating the long-term effect of body fat during childhood on the retinal microvasculature years after. The longitudinal data of body

composition on a 7-year frame with 6 measurement waves is a real asset compared to the literature. Another strength is the use of validated techniques to measure the retinal microvasculature. Herein, a scaling factor was calculated to adjust for the magnification differences, which could be introduced by camera optics, scanner resolution or patient position. In addition, body composition parameters were based not only on BMI but also on advanced body fat measurements (BOD POD^{TM} device) and by considering the waist circumference as marker of central adiposity.

Still, some limitations need to be mentioned. Firstly, the lack of longitudinal data for the retinal photographs (only measured in the last survey) limits us in clarifying the relation between body fat and retinal indicators, but it does impose new starting points for research. Secondly, some selection bias might be present with mainly low-BMI children. Thirdly, random misclassification might happen due to vasomotion of the vessels. In the sensitivity analysis we therefore only included children with two retinal photographs (right and left eye), which confirmed the presented results. Fourthly, we could not correct for iris color, as this information was not available. Finally, we adjusted for all other confounders which we considered important, bus some confounding might still be left.

Conclusion

In our healthy population of school-aged children, higher body fat during childhood was associated with the CRVE, but not the CRAE. The effects were more explicit in boys and at the extremes i.e. in children with overweight or excess adiposity, suggesting an exponential relation between body fat and alterations in the microcirculation. This is of clinical importance since cardiovascular ageing might thus already start during childhood, especially in children with a less favorable body composition. This study suggests that the retinal microvasculature could be a useful tool in identifying children who are at future risk of cardio- or cerebro-vascular diseases. Further studies should focus on changes in the retinal microvasculature over time in relation to body fat and study whether these changes can be reversed by targeted intervention programs.

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Conflict of interest

The authors declare no conflict of interest.

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Tables and figures

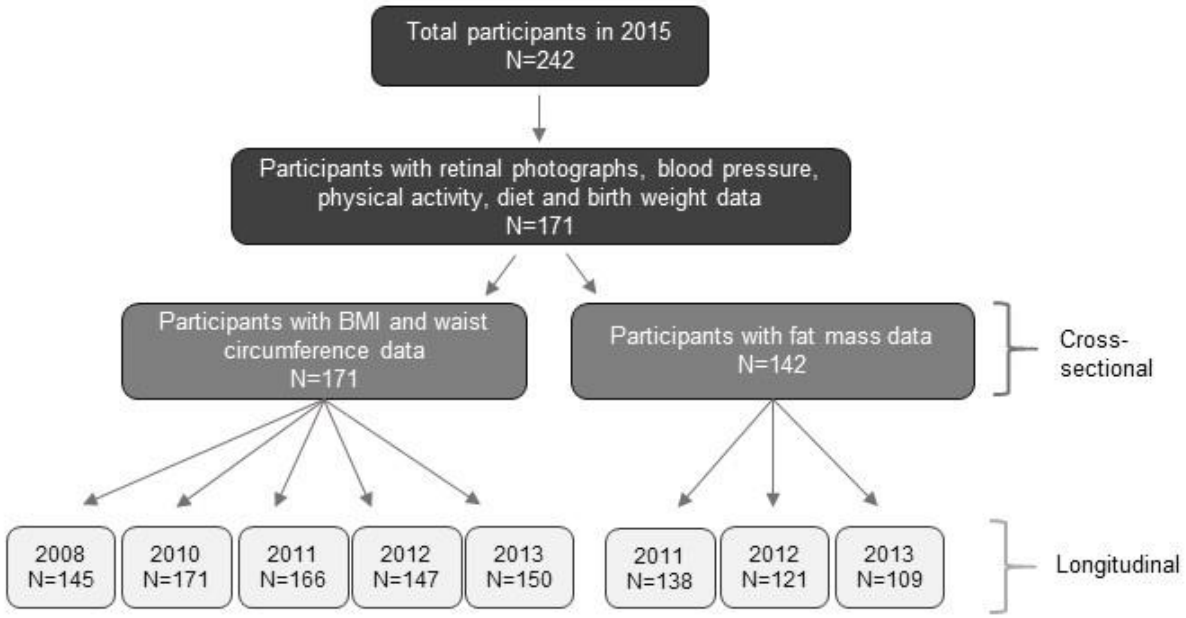

Figure 1. Flow diagram of inclusion and exclusion of study participants. Abbreviations: BMI; body mass index.

Table 1. Characteristics of the study population per study wave.

	Study wave					
	2008	2010	2011	2012	2013	2015
	(N=145)	(N=171)	(N=166)	(N=147)	(N=150)	(N=171)
Age (year)	5.3 (2.7-8.1)	7.5 (4.7-10.3)	8.4 (5.7-11.2)	9.4 (6.7-12.2)	10.5 (7.9-13.4)	12.4 (9.7-15.2)
No males (%)	78 (53.8)	92 (53.8)	90 (54.2)	79 (53.7)	83 (55.3)	92 (53.8)
BMI (kg m^{-2})	15.2 (1.1)	15.4 (1.3)	15.8 (1.6)	16.2 (1.8)	16.3 (1.8)	17.5 (2.4)
zBMI	-0.3 (0.9)	-0.3 (0.8)	-0.3 (0.8)	-0.3 (0.8)	-0.5 (0.8)	-0.4 (0.9)
Overweight (%)	4 (2.8)	5 (2.9)	5 (3.0)	7 (4.8)	2 (1.3)	7 (4.1)
WC (cm)	51.3 (3.7)	54.9 (4.4)		58.5 (5.8)	59.6 (5.6)	62.8 (6.3)
zWC	-0.1 (0.9)	-0.0 (0.9)		-0.0 (0.9)	-0.1 (0.9)	-0.1 (0.9)
zFMI			-0.4 (0.8)	-0.5 (0.9)	-0.7 (0.9)	-0.4 (1.0)
MAP (mmHg)						78.5 (5.4)
Physical activity (hours/week)						10.2 (6.4)
Fat propensity ratio						31.6 (8.1)
Sugar propensity ratio						27.3 (12.4)
Birth weight (kg)						3.4 (0.5)
CRAE (μm)						165.2 (12.5)
CRVE (μm)						223.3 (16.1)
AVR						0.7 (0.0)

Abbreviations: BMI, body mass index; zBMI, age and sex-adjusted z score of body mass index; WC, waist circumference; zWC, age and sex-adjusted z score of waist circumference; zFMI, age and sex-adjusted z score of fat mass index; MAP, mean arterial pressure; CRAE central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriolar to venular ratio.

Values are expressed as medians (minimum-maximum), numbers (percentages) or means (SD).

Table 2. Cross-sectional association between anthropometry (BMI, WC, FMI) and retinal vessel caliber in 2015.

			CRAE (µm)		*CRVE (µm)*		*AVR*	
	Sex	*N*	*B (95% CI)*	*P*	*B (95% CI)*	*P*	*B (95% CI)*	*P*
zBMI		171	-0.323 (-2.048; 1.402)	0.712	1.000 (-1.281; 3.280)	0.388	-0.003 (-0.011; 0.006)	0.512
zWC		171	-0.101 (-1.859; 1.657)	0.910	0.675 (-1.653; 3.003)	0.568	-0.002 (-0.010; 0.007)	0.721
zFMI	Male	70	-1,783 (-3.879; 0.313)	0.094	2.451 (-0.799; 5.701)	0.137	-0.010 (-0.021; 0.002)	0.098
	Female	72	0.435 (-2.272; 3.143)	0.749	-0.710 (-4.041; 2.621)	0.672	0.003 (-0.010; 0.015)	0.680

Abbreviations: CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriolar to venular ratio; CI, confidence interval; B, unstandardized coefficient; zBMI, age and sex-adjusted z score of body mass index; zWC, age and sex-adjusted z score of waist circumference; zFMI, age and sex-adjusted z score of fat mass index.

Linear regression models controlled for age, sex, mean arterial pressure, other retinal parameter (CRAE in case of CRVE and vice versa), physical activity, fat propensity ratio, sugar propensity ratio and birth weight

Table 3. Cross-sectional association between excess adiposity (based on zBMI, zWC and zFMI) and retinal vessel calibers in 2015.

				CRAE (µm)		*CRVE (µm)*		*AVR*	
	Sex		*N*	*B (95% CI)*	*P*	*B (95% CI)*	*P*	*B (95% CI)*	*P*
zBMI		Normal	122	Ref		Ref		Ref	
		Underweight	42	0.867 (-2.745; 4.480)	0.636	-2.121 (-6.858; 2.616)	0.378	0.007 (-0.011; 0.024)	0.456
		Overweight	7	0.859 (-7.010; 8.729)	0.830	8.223 (-2.036; 18.482)	0.115	-0.012 (-0.050; 0.026)	0.534
zWC		Normal	155	Ref		Ref		Ref	
		Excess adiposity	16	-2.745 (-7.956; 2.467)	0.300	6.026 (-0.808; 12.859)	0.084	-0.020 (-0.045; 0.006)	0.128
zFMI	Male	Normal	50	Ref		Ref		Ref	
		Excess adiposity	20	-0.930 (-7.489; 1.629)	0.204	**8.098 (1.563; 14.631)**	**0.016**	**-0.026 (-0.049; -0.002)**	**0.032**
	Female	Normal	53	Ref		Ref		Ref	
		Excess adiposity	19	-2.051 (-7.700; 3.597)	0.471	-0.272 (-7.288; 6.744)	0.939	-0.006 (-0.032; 0.021)	0.669

Abbreviations: CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriolar to venular ratio; CI, confidence interval; B, unstandardized coefficient; Ref, reference category; zBMI, age and sex-adjusted z score of body mass index; zWC, age and sex-adjusted z score of waist circumference; zFMI, age and sex-adjusted z score of fat mass index.

Linear regression models controlled for age, sex, mean arterial pressure, other retinal parameter (CRAE in case of CRVE and vice versa), physical activity, fat propensity ratio, sugar propensity ratio and birth weight.

Table 4. Change in anthropometry (BMI, WC, FMI) over time as predictor of retinal vessel caliber in 2015.

				CRAE (µm)			*CRVE (µm)*			*AVR*		
	Time period	*Sex*	*N*	*B (95% CI)*	*P*	R^2	*B (95% CI)*	*P*	R^2	*B (95% CI)*	*P*	R^2
ΔzBMI[1]	2008-2015		145	-0.025 (-2.350; 2.300)	0.983	0.407	0.263 (-2.770; 3.295)	0.864	0.369	0,000 (-0.011; 0.012)	0.942	0.060
	2010-2015		171	-0.526 (-3.571; 2.520)	0.734	0.372	-0.056 (-4.093; 3.982)	0.978	0.343	0.000 (-0.015; 0.015)	0.985	0.034
	2011-2015		166	-1.193 (-4.656; 2.270)	0.497	0.376	2.519 (-2.003; 7.041)	0.273	0.353	-0.006 (-0.023; 0.011)	0.457	0.051
	2012-2015		147	-0.294 (-4.392; 3.803)	0.887	0.370	2.963 (-2.271; 8.196)	0.265	0.366	-0.004 (-0.024; 0.015)	0.660	0.028
	2013-2015		150	0.556 (-3.903; 5.014)	0.806	0.375	3.935 (-1.640; 9.510)	0.165	0.381	-0.003 (-0.024; 0.017)	0.741	0.047
ΔzWC[2]	2008-2015		139	-1.013 (-3.232; 1.206)	0.368	0.405	1.128 (-1.785; 4.040)	0.445	0.365	-0.005 (-0.016; 0.006)	0.386	0.067
	2010-2015		171	0.174 (-1.905; 2.253)	0.869	0.372	0.420 (-2.335; 3.175)	0.764	0.342	0.001 (-0.010; 0.011)	0.909	0.032
	2012-2015		147	0.739 (-1.795; 3.272)	0.565	0.371	-0,422 (-3.696; 2.853)	0.799	0.359	0.003 (-0.009; 0.015)	0.628	0.028
	2013-2015		96	-0.084 (-3.638; 3.470)	0.963	0.411	-1.999 (-6.383; 2.385)	0.367	0.386	0.003 (-0.013; 0.020)	0.696	0.071
ΔzFMI[3]	2011-2015	Male	68	0.039 (-4.042; 4.120)	0.985	0.388	**7.260 (1.663; 12.857)**	**0.012**	**0.357**	-0.015 (-0.035; 0.004)	0.125	0.070
		Female	70	-0.744 (-4.967; 3.479)	0.736	0.534	1.026 (-4.287; 6.338)	0.701	0.511	-0.002 (-0.022; 0.018)	0.857	0.199
	2012-2015	Male	59	-0.146 (-3.524; 3.233)	0.931	0.440	**5.447 (1.414; 9.480)**	**0.009**	**0.509**	-0.010 (-0.025; 0.005)	0.192	0.197
		Female	62	2.927 (-1.866; 7.720)	0.226	0.494	-1.339 (-7.476; 4.798)	0.663	0.485	0.013 (-0.010; 0.035)	0.269	0.132
	2013-2015	Male	53	0.044 (-4.017; 4.106)	0.983	0.375	**6.565 (1.650; 11.479)**	**0.010**	**0.432**	-0.012 (-0.030; 0.006)	0.195	0.125
		Female	56	1.860 (-3.059; 6.779)	0.450	0.568	-2.366 (-8.113; 3.381)	0.412	0.536	0.010 (-0.012; 0.033)	0.352	0.233

Abbreviations: CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriolar to venular ratio; CI, confidence interval; R^2, adjusted R-squared; B, unstandardized coefficient; zBMI, age and sex-adjusted z score of body mass index; zWC, age and sex-adjusted z score of waist circumference; zFMI, age and sex-adjusted z score of fat mass index.

[1]Linear regression models controlled for age, sex, zBMI (2015), mean arterial pressure, other retinal parameter (CRAE in case of CRVE and vice versa), physical activity, fat propensity ratio, sugar propensity ratio and birth weight.

[2]Linear regression models controlled for age, sex, zWC (2015), mean arterial pressure, other retinal parameter (CRAE in case of CRVE and vice versa), physical activity, fat propensity ratio, sugar propensity ratio and birth weight.

[3]Linear regression models controlled for age, sex, zFMI (2015), mean arterial pressure, other retinal parameter (CRAE in case of CRVE and vice versa), physical activity, fat propensity ratio, sugar propensity ratio and birth weight.

Supplementary information

Regression models on a subset of participants, to control for age, sex, mean arterial pressure and the other retinal parameter.

Table S1. Cross-sectional association between anthropometry (BMI, WC, FMI) and retinal vessel caliber in 2015.

			CRAE (µm)		*CRVE (µm)*		*AVR*	
	Sex	*N*	*B (95% CI)*	*P*	*B (95% CI)*	*P*	*B (95% CI)*	*P*
zBMI		218	-0.023 (-1.505; 1.458)	0.975	1.387 (-0.556; 3.330)	0.161	-0.002 (-0.010; 0.005)	0.502
zWC		218	0.230 (-1.220; 1.680)	0.755	0.715 (-1.200; 2.630)	0.463	-0.000 (-0.008; 0.007)	0.899
zFMI	Male	91	-0.699 (-2.669; 1.271)	0.483	1.512 (-1.249; 4.272)	0.280	-0.005 (-0.015; 0.006)	0.364
	Female	92	1.323 (-1.052; 3.698)	0.271	-0.947 (-3.951; 2.057)	0.533	0.006 (-0.005; 0.017)	0.306

Abbreviations: CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriolar to venular ratio; CI, confidence interval; B, unstandardized coefficient; zBMI, age and sex-adjusted z score of body mass index; zWC, age and sex-adjusted z score of waist circumference; zFMI, age and sex-adjusted z score of fat mass index.
Linear regression models controlled for age, sex, mean arterial pressure and other retinal parameter (CRAE in case of CRVE and vice versa).

Table S2. Cross-sectional association between excess adiposity (based on zBMI, zWC and zFMI) and retinal vessel calibers in 2015.

				CRAE (µm)		*CRVE (µm)*		*AVR*	
	Sex		*N*	*B (95% CI)*	*P*	*B (95% CI)*	*P*	*B (95% CI)*	*P*
zBMI		Normal	157	Ref		Ref		Ref	
		Underweight	53	-0.699 (-3.805; 2.407)	0.658	-1.173 (-5.227; 2.880)	0.569	-0.000 (-0.016; 0.015)	0.965
		Overweight	8	0.770 (-6.334; 7.874)	0.831	**11.000 (1.845; 20.155)**	**0.019**	-0.018 (-0.052; 0.016)	0.296
zWC		Normal	195	Ref		Ref		Ref	
		Excess adiposity	23	-1.111 (-5.449; 3.227)	0.614	4.986 (-0.682; 10.655)	0.084	-0.013 (-0.034; 0.008)	0.237
zFMI	Male	Normal	66	Ref		Ref		Ref	
		Excess adiposity	25	-0.441 (-4.655; 3.774)	0.836	5.452 (-0.265; 11.169)	0.061	-0.012 (-0.034; 0.009)	0.259
	Female	Normal	68	Ref		Ref		Ref	
		Excess adiposity	24	1.481 (-3.320; 6.283)	0.541	-2.138 (-8.147; 3.869)	0.481	0.008 (-0.015; 0.030)	0.502

Abbreviations: CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriolar to venular ratio; CI, confidence interval; B, unstandardized coefficient; Ref, reference category; zBMI, age and sex-adjusted z score of body mass index; zWC, age and sex-adjusted z score of waist circumference; zFMI, age and sex-adjusted z score of fat mass index.
Linear regression models are adjusted for age, sex, mean arterial pressure and other retinal parameter (CRAE in case of CRVE and vice versa).

Table S3. Change in anthropometry (BMI, WC, FMI) over time as predictor of retinal vessel caliber in 2015**.**

				CRAE (µm)			*CRVE (µm)*			*AVR*		
	Time period	*Sex*	*N*	*B (95% CI)*	*P*	R^2	*B (95% CI)*	*P*	R^2	*B (95% CI)*	*P*	R^2
ΔzBMI[1]	2008-2015		163	0.742 (-1.329; 2.813)	0.480	0.448	0.198 (-2.601; 2.998)	0.889	0.387	0.003 (-0.007; 0.013)	0.593	0.076
	2010-2015		195	0.333 (-2.268; 2.934)	0.801	0.410	-0.233 (-3.713; 3.247)	0.895	0.369	0.003 (-0.010; 0.016)	0.638	0.046
	2011-2015		190	-0.737 (-3.758; 2.285)	0.631	0.411	2.612 (-1.375; 6.599)	0.198	0.374	-0.005 (-0.020; 0.010)	0.499	0.054
	2012-2015		169	0.111 (-3.257; 3.478)	0.948	0.412	2.360 (-2.048; 6.767)	0.292	0.380	-0.002 (-0.018; 0.014)	0.813	0.028
	2013-2015		197	0.873 (-2.644; 4.390)	0.265	0.399	5.405 (-1.034; 7.844)	0.132	0.380	-0.002 (-0.019; 0.014)	0.798	0.042
ΔzWC[2]	2008-2015		157	-0.502 (-2.427; 1.422)	0.607	0.438	1.405 (-1.174; 3.984)	0.283	0.386	-0.004 (-0.013; 0.006)	0.437	0.079
	2010-2015		195	0.319 (-1.512; 2.150)	0.732	0.411	1.013 (-1.433; 3.460)	0.415	0.369	0.000 (-0.009; 0.009)	0.999	0.044
	2012-2015		169	0.601 (-1.601; 2.803)	0.591	0.412	-0.090 (-2.994; 2.814)	0.951	0.376	0.003 (-0.009; 0.013)	0.679	0.028
	2013-2015		136	-1.446 (-4.136; 1.243)	0.289	0.420	0.989 (-2.384; 4.361)	0.563	0.382	-0.006 (-0.019; 0.007)	0.349	0.046
ΔzFMI[3]	2011-2015	Male	78	-0.853 (-3.927; 2.222)	0.582	0.376	**4.543 (0.473; 8.614)**	**0.029**	**0.338**	-0.011 (-0.027; 0.004)	0.146	0.030
		Female	81	0.298 (-3.598; 4.194)	0.879	0.475	0.816 (-4.237; 5.869)	0.749	0.472	0.002 (-0.017; 0.020)	0.858	0.081
	2012-2015	Male	68	0.039 (-2.935; 3.013)	0.979	0.384	**4.496 (0.905; 8.087)**	**0.015**	**0.398**	-0.008 (-0.022; 0.006)	0.257	0.026
		Female	72	1.856 (-2.540; 6.252)	0.402	0.469	-0.791 (-6.601; 5.019)	0.787	0.473	0.009 (-0.012; 0.030)	0.410	0.062
	2013-2015	Male	70	0.743 (-2.652; 4.138)	0.664	0.348	**4.510 (0.238; 8.783)**	**0.039**	**0.368**	-0.006 (-0.022; 0.010)	0.459	0.020
		Female	71	1.186 (-3.494; 5.865)	0.615	0.498	-1.926 (-7.342; 3.491)	0.480	0.483	0.008 (-0.013; 0.029)	0.459	0.119

Abbreviations: CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriolar to venular ratio; CI, confidence interval; R^2, adjusted R-squared; B, unstandardized coefficient; zBMI, age and sex-adjusted z score of body mass index; zWC, age and sex-adjusted z score of waist circumference; zFMI, age and sex-adjusted z score of fat mass index.

[1]Linear regression models controlled for age, sex, zBMI (2015), mean arterial pressure and other retinal parameter (CRAE in case of CRVE and vice versa).

[2]Linear regression models controlled for age, sex, zWC (2015), mean arterial pressure and other retinal parameter (CRAE in case of CRVE and vice versa).

[3]Linear regression models controlled for age, sex, zFMI (2015), mean arterial pressure and other retinal parameter (CRAE in case of CRVE and vice versa).