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Longitudinal association between psychosocial stress and retinal microvasculature in children and adolescents

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Highlights

- We performed a 4y longitudinal study in 182 Belgian children (5-11y at start)
- Retinal microvessels were used as proxy of systemic microcirculation
- Psychosocial stress unfavorably affect retinal vascular diameters, not bifurcation
- Stress might thus increase cardio/cerebrovascular disease risk already in children
- Probably independent of lifestyle and overweight

Abstract

Background: Retinal microvessels provides a window to assess the microcirculation of heart and brain, and might reflect cardio- or cerebrovascular disease risk. Limited information exist on the relation between psychosocial stress and the microcirculation, even though psychosocial stress might trigger vascular diseases. This study investigates whether childhood psychosocial stress is a predictor of retinal microvasculature.

Methods: We followed-up 182 Belgian children, aged 5.7-11.3 years at baseline (53.3% boys). Information about psychosocial stress was obtained using emotional, behavioral and negative life events questionnaires and hair cortisol, an objective stress marker. Retinal photographs were used to calculate vessel diameters, bifurcation angles and optimality deviation with semiautomated software. Cross-sectional and longitudinal associations were explored using multivariable regression analysis with retinal parameters in 2015 as outcome, while adjusting for age, sex, socioeconomic status, cardiovascular parameters and lifestyle factors.

Results: Feelings of happiness, sadness and negative life events were associated with retinal vascular diameter, but behavior and hair cortisol were not. High stress levels over a 4-year time period (less

happy, sadder and higher total negative emotions) were associated with larger venules (β= 0.21 - 0.43) and children who experienced more negative life events had smaller arterioles (β= -0.15). No consistent patterns were seen with bifurcation angles and optimality deviation.

Conclusion: Based on the results, we conclude that high levels of childhood psychosocial stress unfavorably affect the retinal vascular diameters, potentially reflecting the microvasculature of the heart and brain. It seems this might even be independent of lifestyle and BMI, but further research on mechanisms is necessary.

Keywords: microvessels, retina, stress, cortisol, behavior, life events, children

1. Introduction

Psychosocial stress is linked to prolonged activation of the allostatic systems, with negative health outcomes, such as cardiovascular disease.(Danese and McEwen, 2012; McEwen, 2007) It is hypothesized that stress can contribute to cardiovascular diseases at different stages, including the long-term development of atherosclerosis and acute triggering of cardiac events.(Bairey Merz et al., 2002; Brotman et al., 2007; Steptoe and Kivimaki, 2012) In addition, the cardiovascular response to stress is different for acute and chronic stress.(Dimsdale, 2008; Steptoe and Kivimaki, 2012) Acute stress mainly triggers acute thrombotic and arrhythmic cardiovascular events, while chronic stress seems to affect cardiovascular risk by accelerating the atherosclerotic process.(Dimsdale, 2008; Steptoe and Kivimaki, 2012) Although stress has been associated with cardiovascular disease risk(Chida and Steptoe, 2010), few studies have examined the associations of stress with microvascular effects, such as changes in retinal vascular diameters, even though the microcirculation constitutes the majority of the circulatory system.

The retinal microvasculature is seen as a good marker to assess the systemic microvasculature: it can be visualized non-invasively and it shares developmental and anatomical similarities with the microcirculation of the heart and brain.(Patton et al., 2005) Changes in the retinal microvasculature may thus reflect similar changes in the cardiovascular and cerebrovascular microvasculature, which might be important in determining an individual's risk on cardiovascular, e.g. hypertension, myocardial infarcts and ischemic heart disease death, or cerebrovascular disease, e.g. vascular dementia and stroke.(Heringa et al., 2013; Liew and Wang, 2011; McClintic et al., 2010; Sun et al., 2009; Witt et al., 2006) Literature shows that cognitive functioning might also be associated with retinal microvascular changes (arteriolar narrowing).(Heringa et al., 2013; Shalev et al., 2013) Besides this, studies have also

reported associations between retinal venular widening and schizophrenia.(Meier et al., 2015; Meier et al., 2013) It is therefore interesting to understand the determinants of the retinal microvasculature.

Unfortunately there is limited information on the relationship between psychosocial stress and retinal microvasculature. To the best of our knowledge only one study exists on this topic. Gerber *et al.* studied how physical fitness can buffer the negative association between psychosocial stress and retinal vessel diameters in first grade primary school children. In this study, a weak association was found between peer stress and wider arterioles.(Gerber et al., 2016) In addition, anxiety and depressive symptoms are two factors that are related to stress and the impact of those factors on retinal vessel diameters have received more attention. These two have been linked to arteriolar narrowing and venular widening in adult populations and one adolescent population.(Cheung et al., 2009; Ikram et al., 2010; Jensen et al., 2010; Kim et al., 2011; Li et al., 2013; Meier et al., 2014)

Besides the study in first grade primary school children no data exists on the effect of psychosocial stress on retinal vessel morphology in children and adolescents. Taken into account that psychosocial stress experienced by children and adolescents may have long-term negative health effects, the aim of this study was to investigate the association between changes in psychosocial stress over 4-year time period (feelings of happiness, sadness, angriness, anxiousness, behavioral problems and negative life events) and metrics related to retinal vessel morphology in a healthy group of Belgian children and adolescents. In addition, this study includes hair cortisol measurements as an objective stress biomarker. We also measured parameters related to the retinal vascular network architecture, e.g. bifurcation angles and bifurcation optimality deviation. Relatively few studies used the latter two markers of the retinal microvasculature, compared to the vascular diameters, whereas both are also found to be related to cardiovascular risk and might therefore be interesting parameters.(Cheung et al., 2011; Witt et al., 2006)

2. Methods

2.1 Study population

In 2015, 242 Flemish children and adolescents participated in the sixth wave of a 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.(Ahrens et al., 2011; Michels et al., 2012) For the current article we used data from 2011 onwards, because of incomplete stress questionnaires before 2011. We included children based on the availability of retinal photographs and stress data in 2015, as can be seen in Figure 1. After exclusion of participants without retinal photographs (N=23), parental socioeconomic status data (N=12) and incomplete stress questionnaires in 2015 (N=2), this resulted in 205 subjects in 2015, of all with Caucasian ethnicity, except one participant from African origin. Children with hair cortisol data is a subset (N=137) in the study because the collection of a hair strand was an optional part of the survey and sometimes hair was not long enough for cortisol analysis. The subset did not differ from the total population, however in general more females were able to donate a hair strand compared to boys. 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.

2.2 Psychosocial stress

Four stress related tests were used to assess a child's psychosocial stress. First, children were questioned about recent feelings of happiness, sadness, angriness and anxiousness using a 0 to 10 Likert scale, with 0 as lowest and 10 as highest score. Total negative emotions were obtained by adding

up the negative emotions sadness, angriness and anxiousness. Second, to assess children's behavioural problems over the past 6 months parents filled in the Strengths and Difficulties Questionnaire (SDQ) (Cronbach's alpha=0.53-0.76, test-retest stability r=0.88, concurrent validity r=0.7-0.87).(Goodman, 1997) The SDQ consisted of 25 questions which can be divided in five subscales (each 5 items): conduct problems, hyperactivity problems, emotional problems, peer relationship problems and prosocial behaviour.(Goodman, 1997) A general total difficulty score was calculated by adding up all subscales except the prosocial behaviour scale (since this is a strength). Thirdly, the Coddington Life Events Scale for children (CLES-C; test-retest r=0.69, parent-child agreement ICC=0.45) measured the frequency and timing of negative events in the last 12 months, such as familiar issues (e.g. divorce), school issues (e.g. failing a grade), social issues (e.g. moving), criminal issues, economical issues (e.g. job loss of a parent) and illness/death of child, family, friend or pet, reported by the children.(Coddington, 1972) This resulted in a 'life change units' score of the frequency and timing of negative events in the last year. Last, in addition to the stress questionnaires, hair cortisol was used as an objective stress biomarker in 2015 only (higher cortisol corresponds with higher stress).(Wester and van Rossum, 2015) A hair strand with a diameter of 3-5 mm was cut from the back of the scalp, closest to the scalp. The proximal 3 cm of the strand was analysed, which reflects stress exposure during the last three months. Extraction and liquid chromatography coupled with tandem mass spectrometry was performed at the Laboratory for Hormonology, Ghent University Hospital Belgium. For analysis on 15 mg hair, inter-assay CV for cortisol is 10.8% with an LOQ of 1.6 pg/mg hair. Detailed laboratory analyses are described elsewhere.(Michels et al., 2016) None of the participants took systemic corticosteroids.

2.3 Retinal microvasculature: vessel diameter, bifurcation angles and bifurcation optimality deviations

Retinal photographs (Canon 45° 6.3-megapixel digital nonmydriatic retinal camera) were taken from both eyes in 2015 only. The retinal vessel measurement system IVAN (University of Wisconsin, Madison, Wisconsin, USA) was used to measure retinal vessel diameters by one trained grader. A

scaling factor was calculated before 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. 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 of Knudtson *et al.*(Knudtson et al., 2003) The arteriolar-to-venular ratio (AVR) depicts the ratio between the CRAE and CRVE. Photographs of both eyes, if present (N=181), were used to estimate the retinal microvasculature. In case only one photograph was available (N=24), this photograph was used to calculate the aforementioned microvascular indexes.

Arteriolar and venular bifurcation angles and bifurcations optimality deviations were calculated using Iflexis computer software (VITO, Mol, Belgium). Bifurcation angles were measured as the angle between daughter vessels (D_1 and D_2) that bisect the parent vessel (D_0). The relationship between the parent and daughter diameters was quantified by the optimality ratio, a measure of equitability of distribution of flow from parent to daughter vessels. The optimality ratio was calculated from the parent diameter (D_0) and daughter vessel diameters (D_1 and D_2) using formulas proposed by Prabhakar *et al*.(Prabhakar et al., 2015) Finally, we defined the optimality deviation, a measure of the extent of deviation from the optimum bifurcation conditions as defined by Murray's law.(Murray, 1926; Witt et al., 2010)

2.4 Covariates

Covariates were obtained during the last follow-up survey. Parental socioeconomic status (SES) was estimated, based on the parent with the highest achieved education, according to the International Standard Classification of Education (ISCED).(UNESCO, 2010) Due to low numbers of participants in category zero to four, the ISCED-categories were combined into two levels (levels 0-4 and levels 5-6). To adjust for temporal changes in blood pressure, which may affect the retinal microvasculature, the

systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured with an automated device (Welch Allyn, USA). Blood pressure was measured twice, with a five minute resting period before the first measurement and a two minute resting period between the measurements. If the two measurements differed more than 5%, a third measurement was carried out. 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). Children's height (m) and weight (kg) were measured to calculate body mass index (BMI), by dividing weight with height squared (kg/m2). The standard deviation score of BMI (zBMI) was computed using the Flemish growth reference data of 2004, to adjust for age and sex.(Roelants et al., 2009) Physical activity was obtained using questionnaires filled in by the parents, 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.*(Lanfer et al., 2012) The average sleep duration during the week was estimated based on reported hours of sleep on weekdays and weekends by parents.

2.5 Statistical analyses

Statistical analyses were performed using statistical software package SAS version 9.4 (SAS Institute Inc, Cary, NC, USA) and all p values <0.05 were considered significant. Hair cortisol concentrations were converted to the natural logarithm. Paired t-test was used to assess individual change over 2011 and 2015 for normally distributed stress parameters and Wilcoxon signed-rank test for not normally distributed variables. Independent t-test and Mann-Whitney U test were conducted to estimate sex differences in stress and retinal microvasculature. Spearman correlation coefficients were calculated between stress parameters and the metrics of retinal vessel morphology in 2015. Cross-sectional associations were explored using linear regression models, with retinal microvasculature as outcome, while adjusting for age, sex and SES in model 1. Model 2 additionally adjusted for MAP and alternate

retinal parameter (CRVE adjusted for in models with CRAE and vice versa) and model 3 additionally for zBMI, sleep duration, diet (sugar and fat propensity ratio) and physical activity. Models with hair cortisol were in a final step also adjusted for date of hair cortisol analysis and hair color, but this did not change the results. Finally, linear regression models were performed with the baseline and continuous change in stress parameters between two study surveys as predictor. The same adjustments have been done as in the cross-sectional analyses.

3. Results

3.1 Subject characteristics

In 2015, 205 children participated in the sixth follow-up survey. Table 1 shows the characteristics of the study population per study wave. Sex differences were seen for sadness (p = 0.04) and the SDQ subscales hyperactivity problems (p = 0.02), emotional problems (p = 0.04) and prosocial behavior (p = 0.049). Scores were higher in girls, except for hyperactivity problems. Also the CRAE was higher in girls (p < 0.01), compared to boys, whilst no differences were seen with the CRVE and AVR.

Spearman correlations showed associations between stress and retinal vessel metrics. A significant negative correlation was seen between happiness and CRVE (r = -0.14, p = 0.04) and an inverse association between sadness and CRVE (r = 0.16, p = 0.02). Only the prosocial subscale of the SDQ was positively correlated with the CRAE (r = 0.15, p = 0.03). Negative events were negatively correlated with both the CRAE and AVR (r = -0.14, p = 0.048 and r = -0.15, p = 0.03 respectively). Hair cortisol was not correlated with retinal metrics. No stress correlations were seen with the arteriolar and venular bifurcation angles. Arteriolar and venular optimality deviation were associated with prosocial behavior (r = 0.17 – 0.18, p = 0.01 – 0.02).

3.2 Cross-sectional associations between stress and retinal microvasculature

Table 2 presents cross-sectional associations between stress parameters and the retinal microvasculature in children. Negative associations were seen between emotion happiness and CRVE in all three models (standardized regression coefficient β = -0.13 to -0.15), a 1.17-1.45% decrease in CRVE was observed for an interquartile range (IQR) increase in happiness. An increase in CRVE was seen with increasing sadness (1.78-2.35% increase per IQR increase in sadness, β = 0.16 to 0.24) and sum of negative emotions (1.01-1.39% increase per IQR increase in negative emotions, β = 0.14 to

0.19). However, associations with the sum of negative emotions were only seen in the first and second model. Negative associations were also observed between AVR and sadness in all models (0.62-0.63% decrease in AVR per IQR increase in sadness, β = -0.16 to -0.17). Negative life events were negatively associated with the CRAE (β = -0.13 to -0.16) and the AVR (β = -0.16 to -0.17), in all three models, a 0.91-1.02% decrease in CRAE and a 0.43-0.44% decrease in AVR were observed for an IQR increase in negative life events. No associations were seen with hair cortisol (Table 2) or with the SDQ subscales (Supplemental Table S1). Arteriolar bifurcation angle was associated with anxiousness and venular bifurcation angle with happiness and hyperactivity problems, but only in the third model (Supplemental Table S2). No significant associations were found for the optimality deviation.

3.3 Associations between change in stress and retinal microvasculature

Longitudinal associations between change in psychosocial stress over 4 years (2011-2015) and retinal microvasculature are shown in Table 3. Overall associations appear to be comparable with the cross-sectional findings. Negative associations were seen between change in happiness and CRVE in all three models (β = -0.21 to -0.30), a 1.43-1.97% decrease in CRVE was observed for an IQR increase in happiness over time. Sadness (1.77-2.74% increase in CRVE per IQR increase in sadness over time, β = 0.28 to 0.43) and the sum of negative emotions (1.42-2.24% increase in CRVE per IQR increase negative emotions over time, β = 0.21 to 0.32) were positively associated with CRVE, however the latter disappeared when additionally adjusting for lifestyle factors. In addition, also associations between sadness and AVR were only observed in the first and second model. A decrease in CRAE was seen with changes in negative life events (1.06-1.11% decrease in CRAE per IQR increase in negative life events over time, β = -0.15 to -0.16), however these disappeared when adjusting for lifestyle factors. Associations between AVR and negative life events were only observed in the first model (β = -0.17). No associations were seen with changes in SDQ subscales (Supplemental Table S3). Changes in happiness and hyperactivity problems over time were associated with venular bifurcation in the third

model only, while a change in emotional problems was related to arteriolar optimality deviation in the first model only (Supplemental Table S4).

Overall similar relations were seen on shorter time periods, namely changes in stress over two (2013-2015) and three years (2012-2015), which are depicted in Supplementary Tables S5 to S8. Here, happiness was positively and sadness negatively associated with the CRVE. Inverse relations for these two emotions were seen with the AVR. Changes in negative emotions were only significant over three years, while changes in negative events were significantly negatively associated with the CRAE on both time periods. The association between changes in negative events and the AVR was only present over two years.

4. Discussion

This study provides unique data on the relationship between changes in psychosocial stress over a 4-year time period and retinal microvasculature in children and adolescents, as marker for cardio- and cerebrovascular disease. It appears that stress is mainly related to the retinal vascular diameter, compared to other aspects of the retinal vascular architectural network (bifurcation angles and bifurcation optimality deviation). Wider venules were associated with negative feelings (sadness and sum of negative emotions), whilst narrower venules were associated with positive feelings (happiness). Negative life events were related to narrower arterioles. No associations were seen with behavioral problems and hair cortisol. Psychosocial stress during childhood might thus already increase cardio- and cerebrovascular disease risk as reflected by wider venules and narrower arterioles.

Longitudinally, emotional feelings over 4-years were associated with retinal vascular diameter. Happiness was associated with venular narrowing, while sadness and the sum of negative emotions were associated with venular widening. Increasing angriness was also significantly associated with increasing venular diameter, however this disappeared after additional adjustment. No associations were seen with anxiousness. In healthy adult populations, depression has been linked to wider arterioles, narrower arterioles and wider venules, depending on the study.(Cheung et al., 2009; Ikram et al., 2010; Jensen et al., 2010; Kim et al., 2011; Li et al., 2013) Similar changes were also seen in an adolescent population, where depression and anxiety were associated with arteriolar widening.(Meier et al., 2014) In our study, sadness and the sum of negative emotions were only associated with venular widening. The study of Gerber *et al.* reported that only peer stress, and not family stress, school stress and critical life events, was weakly associated with larger arteriolar diameters among first grade school-children.(Gerber et al., 2016) Despite the lack of an association with critical life events in the study by Gerber *et al.,* our study noted a negative relationship between negative life events and

arteriolar diameter and a negative association with the ratio (AVR). This difference might be due to the questionnaires used, as the Life Events Checklist (filled in by parents) was used by Gerber *et al.* and the CLES (filled in by children) in this study. Surprisingly, no relations were detected with behavioral problems obtained with the SDQ, nor with its subscale emotional problems despite the possible link with emotional feelings measured with a different questionnaire. This might be because of a potential bias by the person filling in the questionnaires (SDQ were filled in by parents, while emotional feelings were filled in by the children). To our knowledge, this is the first study investigating the association between bifurcation angles and optimality deviation with stress, suggesting that stress might not be a predictor for the distribution of blood across bifurcations.

4.1 Underlying mechanisms

Even though only small deviations were observed, the association between psychosocial stress with retinal microvasculature raises questions into possible biological mechanisms through which this stressor may lead to deviations in retinal microvasculature. Associations were only found with retinal vascular diameters, in contrast to bifurcation angles and optimality deviations, but a potential explanation for this difference is missing. We hypothesize that one possible pathophysiological pathway through which stress might lead to vascular diameter changes might include endothelial dysfunction, as literature shows that chronic stress affects endothelial function(Toda and Nakanishi-Toda, 2011) and there is evidence that endothelial dysfunction could contribute to development of venular widening.(Wong et al., 2006) However, no associations were seen between stress and bifurcation optimality deviation, an indicator of endothelial dysfunction, in this study.(Griffith et al., 1987) Another possible pathway might include psychosocial stress induced inflammation(Hansel et al., 2010), as C-reactive protein is found to be associated with venular widening in school-age children.(Gishti et al., 2015) Finally, it might be an indirect effect of stress on the retinal microvasculature through lifestyle factors. Indeed, adjusting for lifestyle factors (zBMI, diet, physical

activity and sleep) in this study resulted in a loss of some of these associations. Thus, further research is needed into stress pathophysiology on retinal microvasculature.

4.2 Strengths and limitations

Strengths of our study include the long-term measurements of psychosocial stress over a 4-year time period and the extensive techniques to measure different aspects of the retinal microvasculature.

Still, our study does have some limitations. Retinal photographs and hair samples were only taken during the last visit in 2015, whilst psychosocial stress questionnaires were obtained during each follow up. This limits us in detecting any causal relationship between psychosocial stress and retinal microvasculature. In our study we were not able to detect any relation with the objective stress biomarker hair cortisol. However, this may be due to the collection method as hair cortisol levels provide an average stress level over the last 3 months without detecting fluctuating daily changes. Indeed, a previous study focusing on work-related stress showed that a rise in morning cortisol was associated with cardiovascular outcomes.(Chandola et al., 2008) Finally, multiple well-known confounders were adjusted for (cardiovascular risk factors and lifestyle factors), but some confounding may still be left.

4.3 Conclusion

In conclusion, we have shown that psychosocial stress (less happy, sadder, more negative emotions and negative life events) over a 4-year period during childhood is associated with deviations in retinal vascular diameters. Negative emotions (sadness and sum of negative emotions) and negative life events were associated with larger venules, narrower arterioles and smaller arteriolar-to-venular ratio whilst positive emotions (happiness) were associated with narrower venules. Given the link between the retinal microvasculature and those of the heart and brain, stress during childhood might thus

increase clinical cardio- or cerebrovascular disease risk. It seems that this might even be independent of lifestyle and BMI, but further research on mechanisms is necessary.

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Figures

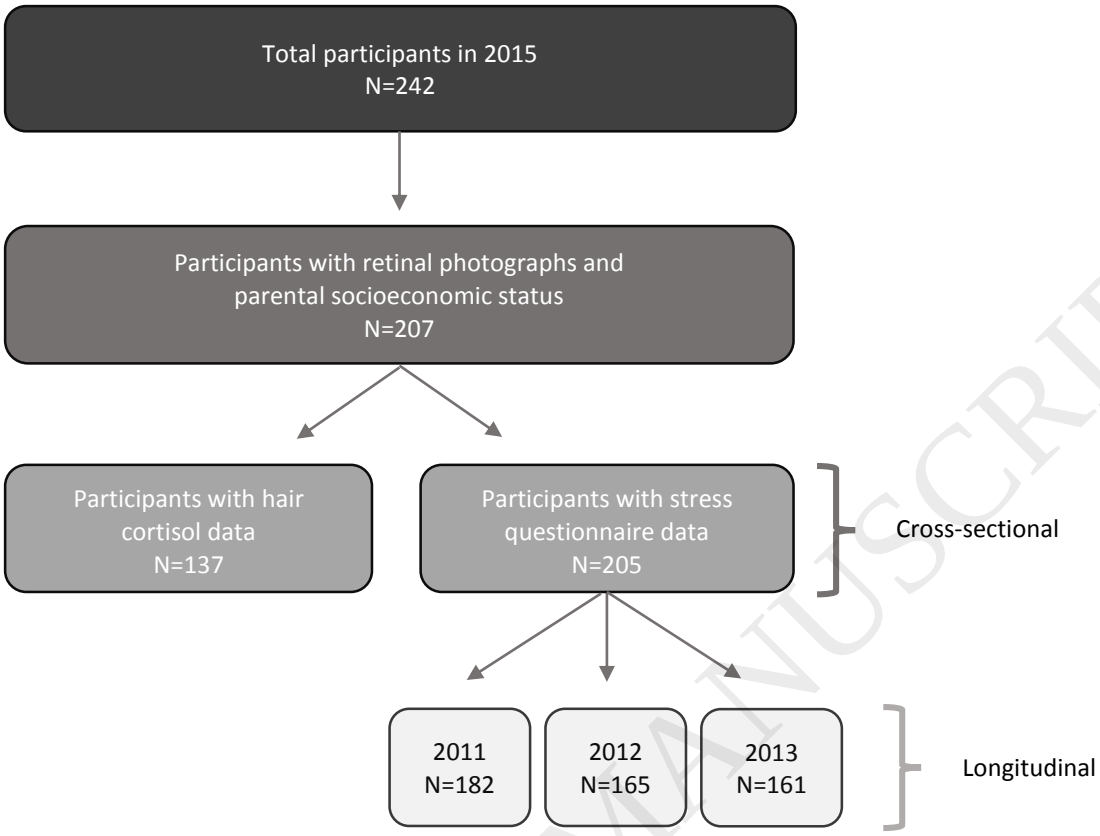

Figure 1. Flow diagram of inclusion and exclusion of study participants.

Tables

Table 1. Characteristics of the study population.

	Study wave				
	2011 (N=182)	2012 (N=165)	2013 (N=161)	2015 (N=205)	*P*-value[$]
Age (year)	8.6 (7.4-9.6)	9.7 (8.4-10.6)	10.6 (9.0-11.7)	12.5 (11.1-13.7)	
Sex, boys (%)	97 (53.3)	87 (52.7)	88 (54.7)	110 (53.1)	
Stress					
Emotions					
Happy (0-10)	8 (7-10)	8 (7-9)	8 (7-10)	8 (7-9)	0.59
Sad (0-10)	2 (0-4)	1 (0-4)	1 (0-3)	1 (0-3)	0.01
Anxious (0-10)	0 (0-2)	0 (0-1)	0 (0-1)	0 (0-2)	0.08
Angry (0-10)	2 (1-4)	2 (1-3)	2 (0-3)	2 (1-3)	0.25
Negative emotions (0-30)	5 (3-10)	5 (2-8)	4 (2-7)	4 (2-7)	0.02
Behaviour					
Conduct problems (0-10)	1 (0-2)	1 (0-2)	1 (0-2)	1 (0-2)	0.17
Hyperactivity problems (0-10)	3 (1-5)	3 (1-4)	3 (1-5)	3 (1-5)	0.69
Emotional problems (0-10)	2 (1-3)	2 (1-3)	2 (0-3)	2 (1-3)	0.42
Peer problems (0-10)	1 (0-2)	1 (0-2)	1 (0-3)	1 (0-2)	0.28
Prosocial behaviour (0-10)	9 (8-10)	9 (8-10)	9 (7-10)	9 (8-10)	0.34
Total difficulties score (0-40)	7 (4-11)	7 (4-11)	8 (5-12)	7 (5-11)	0.54
CLES					
Negative event score last 12 months	47 (0-106)	66 (30-106)	20 (0-96)	56 (18-123)	0.02^
Hair cortisol (pg/mg)*				1.3 (1.0-1.6)	
Retinal microvasculature					
CRAE (µm)				164.6 (158.1-173.0)	
CRVE (µm)				222.6 (211.2-235.2)	
AVR				0.7 (0.7-0.8)	
Arteriolar bifurcation angle (°)				80.5 (72.3-90.7)	
Arteriolar optimality deviation				0.03 (0.00-0.07)	
Venular bifurcation angle (°)				74.0 (64.5-82.4)	
Venular optimality deviation				-0.01 (-0.04-0.01)	
Covariates					
MAP				78.3 (75.5-81.2)	
High SES (N,%)				148 (72.2)	
zBMI				-0.4 (-1.0-0.2)	
Physical activity (hours/week)				9.0 (6.0-14.0)	
Sugar propensity ratio				24.9 (19.8-34.2)	
Fat propensity ratio				30.9 (26.1-36.8)	
Sleep duration (hours/night)				9.6 (9.0-10.3)	

Abbreviations: CLES, Coddington Life Events Scale; CRAE, Central retinal arteriolar equivalent; CRVE, Central retinal venular equivalent; AVR, arteriolar to venular ratio; MAP, mean arterial pressure; SES, parental socioeconomic status; zBMI, age and sex-adjusted z-score of body mass index.

Values are expressed as medians (P25-P75).

*N=137.

[$]Paired t-test *p*-value for individual change over 2011-2015.

^Wilcoxon signed rank test (no normal distribution) for individual change over 2011-2015.

Table 2. Cross-sectional associations between stress (emotions, negative events last 12 months and hair cortisol) and retinal microvasculature in 2015.

		CRAE		CRVE		AVR	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Emotions							
Happy	1	-0.24 (-1.35; 0.87)	0.67	**-1.61 (-3.04; -0.18)**	**0.03**	**0.00 (0.00; 0.01)**	**0.04**
	2	0.43 (-0.46; 1.31)	0.34	**-1.36 (-2.51; -0.22)**	**0.02**	0.00 (-0.00; 0.01)	0.05
	3	0.42 (-0.62; 1.45)	0.43	**-1.44 (-2.77; -0.12)**	**0.03**	0.00 (-0.00; 0.01)	0.08
Sad	1	0.52 (-0.28; 1.33)	0.20	**1.81 (0.78; 2.84)**	**<0.001**	**-0.00 (-0.01; -0.00)**	**0.02**
	2	-0.34 (-0.99; 0.32)	0.31	**1.41 (0.58; 2.23)**	**<0.001**	**-0.00 (-0.00; -0.01)**	**0.02**
	3	-0.46 (-1.22; 0.29)	0.23	**1.20 (0.23; 2.17)**	**0.02**	**-0.00 (-0.01; -0.00)**	**0.04**
Anxious	1	0.20 (-0.84; 1.24)	0.71	0.47 (-0.89; 1.83)	0.49	-0.00 (-0.00; 0.00)	0.71
	2	-0.03 (-0.85; 0.79)	0.95	0.32 (-0.77; 1.40)	0.56	-0.00 (-0.00; 0.00)	0.69
	3	-0.02 (-1.09; 1.04)	0.97	-0.05 (-1.44; 1.35)	0.95	-0.00 (-0.01; 0.01)	0.98
Angry	1	0.48 (-0.48; 1.44)	0.32	1.18 (-0.06; 2.42)	0.06	-0.00 (-0.01; 0.00)	0.36
	2	-0.07 (-0.83; 0.68)	0.85	0.80 (-0.19; 1.80)	0.11	-0.00 (-0.01; 0.00)	0.33
	3	-0.35 (-1.25; 0.54)	0.44	0.53 (-0.64; 1.71)	0.37	-0.00 (-0.01; 0.00)	0.34
Negative emotions	1	0.22 (-0.16; 0.60)	0.26	**0.65 (0.16; 1.14)**	**0.01**	-0.00 (-0.00; 0.00)	0.12
	2	-0.09 (-0.39; 0.22)	0.57	**0.48 (0.09; 0.88)**	**0.02**	-0.00 (-0.00; 0.00)	0.10
	3	-0.18 (-0.56; 0.19)	0.34	0.39 (-0.10; 0.88)	0.12	-0.00 (-0.00; 0.00)	0.15
CLES							
Negative events	1	**-0.02 (-0.03; -0.00)**	**0.04**	0.00 (-0.02; 0.02)	0.96	**-0.00 (-0.00; -0.00)**	**0.02**
	2	**-0.02 (-0.03; -0.01)**	**0.01**	0.02 (-0.00; 0.03)	0.07	**-0.00 (-0.00; -0.00)**	**0.01**
	3	**-0.02 (-0.03; -0.01)**	**0.01**	0.01 (-0.01; 0.03)	0.28	**-0.00 (-0.00; -0.00)**	**0.04**
Hair cortisol (pg/mg)	1	-0.44 (-1.11; 7.95)	0.77	0.17 (-3.74; 4.08)	0.93	-0.00 (-0.01; 0.01)	0.65
	2	-0.43 (-2.73; 1.86)	0.71	0.44 (-2.53; 3.42)	0.77	-0.00 (-0.01; 0.01)	0.70
	3	-2.73 (-5.59; 0.14)	0.06	2.57 (-1.22; 6.36)	0.18	-0.01 (-0.03; 0.00)	0.06

Abbreviations: CRAE, Central retinal arteriolar equivalent; CRVE, Central retinal venular equivalent; AVR, arteriolar to venular ratio; CLES, Coddington Life Events Scale.

Model 1 adjusted for age, sex and socioeconomic status

Model 2 adjusted for age, sex, socioeconomic status, mean arterial pressure and alternate retinal parameter

Model 3 adjusted for age, sex, socioeconomic status, mean arterial pressure, alternate retinal parameter, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity

Table 3. Change in stress (emotions and negative events last 12 months) over time (2011-2015) as predictor of retinal microvasculature in 2015.

		CRAE		CRVE		AVR	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Stress							
Emotions							
ΔHappy	1	-0.56 (-1.78; 0.66)	0.36	**-2.22 (-3.80; -0.64)**	**0.01**	**0.01 (0.00; 0.01)**	**0.03**
	2	0.32 (-0.65; 1.29)	0.52	**-1.64 (-2.91; -0.38)**	**0.01**	0.01 (-0.00; 0.01)	0.06
	3	0.24 (-0.88; 1.37)	0.67	**-1.58 (-3.02; -0.14)**	**0.03**	0.00 (-0.00; 0.01)	0.12
ΔSad	1	0.77 (-0.10; 1.65)	0.08	**2.11 (0.99; 3.23)**	**<0.001**	**-0.00 (-0.01; -0.00)**	**0.04**
	2	-0.25 (-0.95; 0.46)	0.49	**1.51 (0.61; 2.40)**	**0.001**	**-0.00 (-0.01; -0.00)**	**0.03**
	3	-0.37 (-1.18; 0.44)	0.37	**1.34 (0.30; 2.39)**	**0.01**	-0.00 (-0.01; 0.00)	0.05
ΔAnxious	1	0.41 (-0.67; 1.50)	0.45	0.48 (-0.97; 1.93)	0.51	0.00 (-0.00; 0.00)	0.95
	2	0.18 (-0.67; 1.02)	0.68	0.15 (-1.00; 1.31)	0.80	0.00 (-0.00; 0.00)	0.99
	3	0.26 (-0.84; 1.36)	0.64	-0.13 (-1.64; 1.38)	0.86	0.00 (-0.00; 0.01)	0.75
ΔAngry	1	0.54 (-0.47; 1.56)	0.29	**1.36 (0.03; 2.69)**	**0.04**	-0.00 (-0.01; 0.00)	0.31
	2	-0.11 (-0.91; 0.68)	0.78	0.94 (-0.12; 2.00)	0.08	-0.00 (-0.01; 0.00)	0.27
	3	-0.48 (-1.39; 0.43)	0.30	0.64 (-0.60; 1.88)	0.31	-0.00 (-0.01; 0.00)	0.24
ΔNegative emotions	1	0.33 (-0.07; 0.72)	0.11	**0.74 (0.22; 1.27)**	**0.01**	-0.00 (-0.00; 0.00)	0.20
	2	-0.03 (-0.35; 0.29)	0.87	**0.49 (0.07; 0.91)**	**0.02**	-0.00 (-0.00; 0.00)	0.17
	3	-0.14 (-0.53; 0.25)	0.49	0.44 (-0.09; 0.96)	0.10	-0.00 (-0.00; 0.00)	0.17
CLES							
ΔNegative events	1	**-0.02 (-0.04; -0.00)**	**0.01**	-0.00 (-0.03; 0.02)	0.78	**-0.00 (-0.00; -0.00)**	**0.04**
	2	**-0.02 (-0.03; -0.00)**	**0.01**	0.01 (-0.01; 0.03)	0.19	-0.00 (-0.00; 0.00)	0.05
	3	-0.01 (-0.03; 0.00)	0.07	0.00 (-0.01; 0.02)	0.62	-0.00 (-0.00; 0.00)	0.18

Abbreviations: CRAE, Central retinal arteriolar equivalent; CRVE, Central retinal venular equivalent; AVR, arteriolar to venular ratio; CLES, Coddington Life Events Scale.

Model 1 adjusted for age, sex, socioeconomic status and baseline stress parameter

Model 2 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure and alternate retinal parameter

Model 3 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure, alternate retinal parameter, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity

Supplementary information

Longitudinal association between psychosocial stress and retinal microvasculature in children and adolescents

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Table S1. Behavior as predictor of retinal vascular diameters in 2015.

Table S2. Cross-sectional associations between stress (emotions, negative events, hair cortisol and behavior) and retinal network parameters in 2015.

Table S3. Change in behavior over time (2011-2015) as predictor of retinal vascular diameters in 2015.

Table S4. Change in stress (emotions, negative events and behavior) over time (2011-2015) as predictor of retinal network parameters in 2015.

Table S5. Change in stress (emotions, negative events and behavior) over time (2012-2015) as predictor of retinal vascular diameters in 2015.

Table S6. Change in stress (emotions, negative events and behavior) over time (2012-2015) as predictor of retinal network parameters in 2015.

Table S7. Change in stress (emotions, negative events and behavior) over time (2013-2015) as predictor of retinal vascular diameters in 2015.

Table S8. Change in stress (emotions, negative events and behavior) over time (2013-2015) as predictor of retinal network parameters in 2015.

Table S1. Behavior as predictor of retinal vascular diameters in 2015.

		CRAE		CRVE		AVR	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Behavior							
Conduct problems	1	-0.27 (-1.52; 0.97)	0.66	0.28 (-1.35; 1.90)	0.74	-0.00 (-0.01; 0.00)	0.35
	2	-0.52 (-1.49; 0.46)	0.30	0.59 (-0.71; 1.89)	0.37	-0.00 (-0.01; 0.00)	0.24
	3	-0.52 (-1.50; 0.46)	0.30	0.62 (-0.68; 1.92)	0.35	-0.00 (-0.01; 0.00)	0.23
Hyperactivity problems	1	0.13 (-0.67; 0.93)	0.75	0.50 (-0.54; 1.55)	0.34	-0.00 (-0.00; 0.00)	0.47
	2	-0.26 (-0.90; 0.38)	0.43	0.53 (-0.32; 1.37)	0.22	-0.00 (-0.01; 0.00)	0.23
	3	-0.26 (-0.90; 0.38)	0.43	0.57 (-0.27; 1.42)	0.18	-0.00 (-0.01; 0.00)	0.21
Emotional problems	1	-0.07 (-1.01; 0.88)	0.89	0.21 (-1.02; 1.44)	0.74	-0.00 (-0.00; 0.00)	0.51
	2	-0.17 (-0.91; 0.57)	0.65	0.27 (-0.72; 1.25)	0.59	-0.00 (-0.00; 0.00)	0.50
	3	-0.17 (-0.93; 0.58)	0.65	0.36 (-0.64; 1.35)	0.48	-0.00 (-0.01; 0.00)	0.44
Peer problems	1	-0.43 (-1.44; 0.59)	0.41	-0.18 (-1.51; 1.14)	0.78	-0.00 (-0.01; 0.00)	0.49
	2	-0.29 (-1.08; 0.51)	0.48	0.12 (-0.94; 1.18)	0.83	-0.00 (-0.01; 0.00)	0.56
	3	-0.29 (-1.09; 0.51)	0.48	0.13 (-0.93; 1.19)	0.81	-0.00 (-0.01; 0.00)	0.56
Prosocial behavior	1	0.79 (-0.23; 1.82)	0.13	0.59 (-0.75; 1.94)	0.38	0.00 (-0.00; 0.01)	0.42
	2	0.65 (-0.16; 1.46)	0.12	-0.14 (-1.22; 0.95)	0.80	0.00 (-0.00; 0.01)	0.27
	3	0.65 (-0.16; 1.47)	0.12	-0.08 (-1.17; 1.01)	0.88	0.00 (-0.00; 0.01)	0.29
Total difficulties score	1	-0.06 (-0.42; 0.30)	0.74	0.13 (-0.34; 0.59)	0.58	-0.00 (-0.00; 0.00)	0.28
	2	-0.15 (-0.43; 0.13)	0.29	0.20 (-0.17; 0.57)	0.28	-0.00 (-0.00; 0.00)	0.19
	3	-0.15 (-0.44; 0.13)	0.28	0.23 (-0.14; 0.60)	0.22	-0.00 (-0.00; 0.00)	0.16

Abbreviations: CRAE, Central retinal arteriolar equivalent; CRVE, Central retinal venular equivalent; AVR, arteriolar to venular ratio.
Model 1 adjusted for age, sex and socioeconomic status
Model 2 adjusted for age, sex, socioeconomic status, mean arterial pressure and alternate retinal parameter
Model 3 adjusted for age, sex, socioeconomic status, mean arterial pressure, alternate retinal parameter, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity

Table S2. Cross-sectional associations between stress (emotions, negative events, hair cortisol and behavior) and retinal network parameters in 2015.

		Arteriolar bifurcation angle		Arteriolar optimality deviation		Venular bifurcation angle		Venular optimality deviation	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Emotions									
Happy	1	0.92 (-0.68; 2.51)	0.26	-0.00 (-0.01; 0.00)	0.41	0.91 (-0.37; 2.20)	0.16	-0.00 (-0.01; 0.00)	0.36
	2	0.90 (-0.69;2.50)	0.27	-0.00 (-0.01; 0.00)	0.43	0.93 (-0.36; 2.22)	0.16	-0.00 (-0.01; 0.00)	0.39
	3	0.89 (-0.99; 2.77)	0.35	-0.00 (-0.01; 0.00)	0.68	**1.73 (0.25; 3.21)**	**0.02**	-0.00 (-0.01; 0.00)	0.10
Sad	1	-0.04 (-1.13; 1.06)	0.94	-0.00 (-0.00; 0.00)	0.41	-0.15 (-1.04; 0.73)	0.74	0.00 (-0.00; 0.00)	0.45
	2	-0.05 (-1.15; 1.05)	0.93	-0.00 (-0.00; 0.00)	0.43	-0.14 (-1.03; 0.75)	0.76	0.00 (-0.00; 0.00)	0.06
	3	-0.21 (-1.49; 1.06)	0.74	-0.00 (-0.01; 0.00)	0.43	-0.44 (-1.46; 0.57)	0.39	0.00 (-0.00; 0.00)	0.68
Anxious	1	-0.58 (-2.00; 0.83)	0.42	-0.00 (-0.00; 0.00)	0.89	0.03 (-1.12; 1.18)	0.96	0.00 (-0.00; 0.01)	0.42
	2	-0.60 (-2.01l 0.82)	0.41	-0.00 (-0.00; 0.00)	0.90	0.04 (-1.11; 1.19)	0.95	0.00 (-0.00; 0.01)	0.40
	3	**-1.98 (-3.80; -0.16)**	**0.03**	-0.00 (-0.01; 0.00)	0.65	-0.55 (-2.02; 0.93)	0.47	-0.00 (-0.01; 0.00)	0.26
Angry	1	0.44 (-0.84; 1.73)	0.50	-0.00 (-0.01; 0.00)	0.39	-0.12 (-1.16; 0.93)	0.83	0.00 (-0.00; 0.00)	0.42
	2	0.44 (-0.85; 1.73)	0.50	-0.00 (-0.01; 0.00)	0.40	-0.11 (-1.16; 0.94)	0.84	0.00 (-0.00; 0.00)	0.40
	3	0.56 (-0.93; 2.06)	0.46	-0.00 (-0.01; 0.00)	0.22	-0.73 (-1.93; 0.48)	0.24	0.00 (-0.00; 0.00)	0.61
Negative emotions	1	-0.01 (-0.53; 0.50)	0.96	-0.00 (-0.00; 0.00)	0.43	-0.05 (-0.47; 0.37)	0.82	0.00 (-0.00; 0.00)	0.33
	2	-0.02 (-0.54; 0.50)	0.94	-0.00 (-0.00; 0.00)	0.45	-0.04 (-0.46; 0.38)	0.84	0.00 (-0.00; 0.00)	0.30
	3	-0.19 (-0.82; 0.45)	0.57	-0.00 (-0.00; 0.00)	0.28	-0.31 (-0.82; 0.20)	0.24	0.00 (-0.00; 0.00)	0.98
CLES									
Negative events	1	-0.00 (-0.02; 0.02)	0.95	-0.00 (-0.00; 0.00)	0.07	-0.01 (-0.03; 0.01)	0.35	-0.00 (-0.00; 0.00)	0.89
	2	-0.00 (-0.02; 0.02)	0.94	-0.00 (-0.00; 0.00)	0.08	-0.01 (-0.03; 0.01)	0.35	-0.00 (-0.00; 0.00)	0.91
	3	-0.00 (-0.03; 0.02)	0.83	-0.00 (-0.00; 0.00)	0.13	-0.00 (-0.03; 0.01)	0.35	0.00 (-0.00; 0.00)	0.94
Hair cortisol (pg/mg)	1	2.82 (-1.18; 6.82)	0.17	-0.00 (-0.01; 0.01)	0.62	-0.40 (-3.37; 2.56)	0.79	0.00 (-0.01; 0.02)	0.35
	2	2.82 (-1.20; 6.84)	0.17	-0.00 (-0.02; 0.01)	0.61	-0.41 (-3.39; 2.57)	0.79	0.00 (-0.01; 0.02)	0.36
	3	1.23 (-3.87; 6.33)	0.63	-0.01 (-0.02; 0.00)	0.16	-0.37 (-4.14; 3.41)	0.85	0.00 (-0.01; 0.02)	0.49
Behavior									
Conduct problems	1	-0.10 (-1.80; 1.60)	0.91	-0.00 (-0.01; 0.00)	0.45	0.61 (-0.79; 2.01)	0.39	0.00 (-0.00; 0.01)	0.71
	2	-0.13 (-1.84; 1.58)	0.88	-0.00 (-0.01; 0.00)	0.50	0.66 (-0.75; 2.07)	0.35	0.00 (-0.00; 0.01)	0.62
	3	0.08 (-1.81; 1.97)	0.93	0.00 (-0.00; 0.01)	0.88	1.11 (-0.45; 2.67)	0.16	0.00 (-0.00; 0.01)	0.18
Hyperactivity problems	1	-0.20 (-1.29; 0.88)	0.71	-0.00 (-0.01; 0.00)	0.14	0.63 (-0.24; 1.50)	0.15	-0.00 (-0.00; 0.00)	0.58
	2	-0.24 (-1.34; 0.86)	0.66	-0.00 (-0.01; 0.00)	0.18	0.71 (-0.17; 1.58)	0.11	-0.00 (-0.00; 0.00)	0.76

	3	-0.24 (-1.48; 1.00)	0.70	-0.00 (-0.01; 0.00)	0.35	**0.99 (0.01; 1.97)**	**0.048**	0.00 (-0.00; 0.00)	0.61
Emotional problems	1	0.08 (-1.22; 1.38)	0.90	-0.00 (-0.01; 0.00)	0.51	0.05 (-0.99; 1.09)	0.92	-0.00 (-0.00; 0.00)	0.99
	2	0.08 (-1.22; 1.38)	0.90	-0.00 (-0.01; 0.00)	0.51	0.05 (-0.99; 1.09)	0.93	-0.00 (-0.00; 0.00)	0.99
	3	0.09 (-1.36; 1.53)	0.91	-0.00 (-0.00; 0.00)	0.73	0.42 (-0.73; 1.56)	0.47	0.00 (-0.00; 0.01)	0.23
Peer problems	1	0.93 (-0.7; 2.33)	0.19	-0.00 (-0.01; 0.00)	0.62	0.41 (-0.73; 1.55)	0.48	-0.00 (-0.00; 0.00)	0.67
	2	0.95 (-0.45; 2.35)	0.18	-0.00 (-0.01; 0.00)	0.59	0.38 (-0.76; 1.53)	0.51	-0.00 (-0.00; 0.00)	0.61
	3	0.49 (-1.10; 2.10)	0.54	0.00 (-0.00; 0.01)	0.67	0.50 (-0.78; 1.79)	0.44	0.00 (-0.00; 0.00)	0.68
Total difficulties score	1	0.08 (-0.42; 0.57)	0.76	-0.00 (-0.00; 0.00)	0.18	0.25 (-0.16; 0.65)	0.23	-0.00 (-0.00; 0.00)	0.76
	2	0.07 (-0.43; 0.57)	0.78	-0.00 (-0.00; 0.00)	0.21	0.26 (-0.14; 0.66)	0.20	-0.00 (-0.00; 0.00)	0.85
	3	0.03 (-0.53; 0.59)	0.91	-0.00 (-0.00; 0.00)	0.65	0.43 (-0.02; 0.87)	0.06	0.00 (-0.00; 0.00)	0.21
Prosocial behavior	1	-0.06 (-1.46; 1.33)	0.93	0.00 (-0.00; 0.01)	0.06	-0.94 (-2.05; 0.17)	0.10	0.00 (-0.00; 0.01)	0.19
	2	-0.03 (-1.44; 1.37)	0.96	0.00 (-0.00; 0.01)	0.07	-1.01 (-2.12; 0.11)	0.08	0.00 (-0.00; 0.01)	0.25
	3	-0.52 (-2.10; 1.05)	0.51	0.00 (-0.00; 0.01)	0.11	0.21 (-1.87; 0.63)	0.33	0.00 (-0.00; 0.01)	0.14

Abbreviations: CLES, Coddington Life Events Scale.
Model 1 adjusted for age, sex and socioeconomic status
Model 2 adjusted for age, sex, socioeconomic status and mean arterial pressure
Model 3 adjusted for age, sex, socioeconomic status, mean arterial pressure, zBMI, sugar and fat propensity ratio, sleep duration and physical activity.

Table S3. Change in behavior over time (2011-2015) as predictor of retinal vascular diameters in 2015.

		CRAE		CRVE		AVR	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Behavior							
ΔConduct problems	1	-0.51 (-1.86; 0.83)	0.45	0.35 (-1.37; 2.08)	0.69	-0.00 (-0.01; 0.00)	0.20
	2	-0.64 (-1.69; 0.41)	0.23	0.73 (-0.64; 2.11)	0.29	-0.00 (-0.01; 0.00)	0.22
	3	-0.53 (-1.67; 0.61)	0.36	0.99 (-0.51; 2.48)	0.19	-0.00 (-0.01; 0.00)	0.22
ΔHyperactivity problems	1	0.19 (-0.78; 1.15)	0.70	-0.74 (-1.97; 0.49)	0.23	0.00 (-0.00; 0.01)	0.07
	2	0.52 (-0.24; 1.28)	0.18	-0.88 (-1.86; 0.10)	0.08	0.00 (-0.00; 0.01)	0.07
	3	0.31 (-0.55; 1.16)	0.48	-0.70 (-1.81; 0.41)	0.22	0.00 (-0.00; 0.01)	0.26
ΔEmotional problems	1	0.73 (-0.28; 1.74)	0.15	1.20 (-0.09; 2.48)	0.07	-0.00 (-0.00; 0.00)	0.72
	2	0.21 (-0.59; 1.02)	0.60	0.60 (-0.44; 1.65)	0.26	-0.00 (-0.00; 0.00)	0.78
	3	-0.00 (-0.94; 0.93)	0.99	0.48 (-0.75; 1.71)	0.44	-0.00 (-0.01; 0.00)	0.65
ΔPeer problems	1	0.23 (-1.23; 1.69)	0.76	-0.10 (-1.97; 1.78)	0.92	0.00 (-0.00; 0.01)	0.64
	2	0.28 (-0.57; 1.42)	0.63	-0.28 (-1.78; 1.22)	0.71	0.00 (-0.00; 0.01)	0.62
	3	0.13 (-1.20; 1.46)	0.85	-0.92 (-2.66; 0.82)	0.30	0.00 (-0.00; 0.01)	0.50
ΔProsocial behavior	1	-0.44 (-1.98; 1.09)	0.57	-1.11 (-3.08; 0.86)	0.27	0.00 (-0.00; 0.01)	0.55
	2	0.03 (-1.17; 1.24)	0.95	-0.73 (-2.32; 0.85)	0.36	0.00 (-0.00; 0.01)	0.57
	3	-0.19 (-1.52; 1.13)	0.77	-0.92 (-2.66; 0.82)	0.30	0.00 (-0.00; 0.01)	0.63
ΔTotal difficulties score	1	0.12 (-0.31; 0.54)	0.59	0.07 (-0.48; 0.61)	0.81	0.00 (-0.00; 0.00)	0.70
	2	0.10 (-0.24; 0.43)	0.57	-0.03 (-0.47; 0.40)	0.88	0.00 (-0.00; 0.00)	0.65
	3	0.01 (-0.38; 0.39)	0.97	-0.04 (-0.55; 0.46)	0.86	0.00 (-0.00; 0.00)	0.91

Abbreviations: CRAE, Central retinal arteriolar equivalent; CRVE, Central retinal venular equivalent; AVR, arteriolar to venular ratio.
Model 1 adjusted for age, sex, socioeconomic status and baseline stress parameter
Model 2 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure and alternate retinal parameter.
Model 3 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure, alternate retinal parameter, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity

Table S4. Change in stress (emotions, negative events and behavior) over time (2011-2015) as predictor of retinal network parameters in 2015.

		Arteriolar bifurcation angle		Arteriolar optimality deviation		Venular bifurcation angle		Venular optimality deviation	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Emotions									
ΔHappy	1	0.47 (-1.26; 2.20)	0.59	-0.00 (-0.01; 0.00)	0.70	0.57 (-0.85; 2.00)	0.43	-0.00 (-0.01; 0.00)	0.65
	2	0.41 (-1.33; 2.14)	0.64	-0.00 (-0.01; 0.00)	0.78	0.64 (-0.78; 2.06)	0.38	-0.00 (-0.01; 0.00)	0.74
	3	0.65 (-1.40; 2.70)	0.53	0.00 (-0.01; 0.01)	0.99	**1.59 (0.00; 3.19)**	**0.049**	-0.00 (-0.01; 0.00)	0.19
ΔSad	1	0.37 (-0.80; 1.54)	0.54	-0.00 (-0.01; 0.00)	0.20	0.15 (-0.81; 1.11)	0.76	0.00 (-0.00; 0.00)	0.60
	2	0.35 (-0.82; 1.51)	0.56	-0.00 (-0.01; 0.00)	0.21	0.17 (-0.79; 1.13)	0.73	0.00 (-0.00; 0.00)	0.56
	3	0.13 (-1.26; 1.51)	0.85	-0.00 (-0.01; 0.00)	0.26	0.03 (-1.06; 1.11)	0.96	0.00 (-0.00; 0.00)	0.78
ΔAnxious	1	-0.48 (-1.94; 0.98)	0.52	-0.00 (-0.01; 0.00)	0.52	0.19 (-1.01; 1.39)	0.76	0.00 (-0.00; 0.01)	0.47
	2	-0.50 (-1.96; 0.96)	0.50	-0.00 (-0.01; 0.00)	0.54	0.20 (-1.00; 1.40)	0.74	0.00 (-0.00; 0.01)	0.44
	3	-1.73 (-3.64; 0.19)	0.08	-0.00 (-0.01; 0.00)	0.34	-0.09 (-1.63; 1.44)	0.90	-0.00 (-0.01; 0.00)	0.24
ΔAngry	1	0.92 (-0.43; 2.26)	0.18	-0.00 (-0.01; 0.00)	0.49	0.04 (-1.07; 1.14)	0.95	0.00 (-0.00; 0.01)	0.33
	2	0.91 (-0.44; 2.25)	0.19	-0.00 (-0.01; 0.00)	0.51	0.05 (-1.05; 1.15)	0.93	0.00 (-0.00; 0.01)	0.31
	3	0.85 (-0.72; 2.42)	0.29	-0.00 (-0.01; 0.00)	0.23	-0.60 (-1.84; 0.63)	0.33	0.00 (-0.00; 0.01)	0.47
ΔNegative emotions	1	0.15 (-0.38; 0.69)	0.58	-0.00 (-0.00; 0.00)	0.26	0.06 (-0.38; 0.51)	0.78	0.00 (-0.00; 0.00)	0.38
	2	0.15 (-0.39; 0.68)	0.59	-0.00 (-0.00; 0.00)	0.28	0.07 (-0.37; 0.51)	0.76	0.00 (-0.00; 0.00)	0.35
	3	-0.014 (-0.69; 0.66)	0.97	-0.00 (-0.00; 0.00)	0.16	-0.13 (-0.67; 0.40)	0.62	0.00 (-0.00; 0.00)	0.97
CLES									
ΔNegative events	1	-0.00 (-0.02; 0.02)	0.96	-0.00 (-0.00; 0.00)	0.17	-0.01 (-0.03; 0.01)	0.21	-0.00 (-0.00; 0.00)	0.90
	2	-0.00 (-0.02; 0.02)	0.98	-0.00 (-0.00; 0.00)	0.15	-0.01 (-0.03; 0.01)	0.20	-0.00 (-0.00; 0.00)	0.86
	3	-0.00 (-0.03; 0.02)	0.90	-0.00 (-0.00; 0.00)	0.10	-0.01 (-0.03; 0.01)	0.30	-0.00 (-0.00; 0.00)	0.66
Behavior									
ΔConduct problems	1	-0.31 (-2.27; 1.65)	0.75	-0.00 (-0.01; 0.01)	0.97	0.01 (-1.59; 1.61)	0.99	0.00 (-0.00; 0.01)	0.76
	2	-0.39 (-2.35; 1.58)	0.70	0.00 (-0.01; 0.01)	0.93	0.09 (-1.51; 1.69)	0.91	0.00 (-0.00; 0.01)	0.65
	3	-0.30 (-2.51; 1.92)	0.79	0.00 (-0.00; 0.01)	0.31	0.23 (-1.50; 1.96)	0.79	0.00 (-0.00; 0.00)	0.53
ΔHyperactivity problems	1	0.40 (-1.08; 1.87)	0.59	-0.00 (-0.01; 0.00)	0.11	0.70 (-0.49; 1.89)	0.25	-0.00 (-0.01; 0.00)	0.19
	2	0.34 (-1.14; 1.82)	0.65	-0.00 (-0.01; 0.00)	0.14	0.77 (-0.42; 1.96)	0.20	-0.00 (-0.01; 0.00)	0.24
	3	0.21 (-1.50; 1.91)	0.81	-0.00 (-0.01; 0.00)	0.47	**1.58 (0.27; 2.89)**	**0.02**	-0.00 (-0.01; 0.00)	0.55
ΔEmotional problems	1	0.72 (-0.80; 2.23)	0.35	**-0.00 (-0.01; -0.00)**	**0.049**	0.53 (-0.69; 1.75)	0.39	0.00 (-0.00; 0.00)	0.93
	2	0.70 (-0.82; 2.22)	0.36	-0.00 (-0.01; 0.00)	0.05	0.54 (-0.67; 1.76)	0.38	0.00 (-0.00; 0.00)	0.91

	3	0.34 (-1.47; 2.15)	0.71	-0.00 (-0.01; 0.01)	0.66	1.10 (-0.29; 2.49)	0.12	0.00 (-0.00; 0.01)	0.64
ΔPeer problems	1	1.46 (-0.32; 3.25)	0.11	-0.00 (-0.01; 0.00)	0.82	0.60 (-0.87; 2.06)	0.43	-0.00 (-0.01; 0.00)	0.28
	2	1.43 (-0.36; 3.22)	0.12	-0.00 (-0.01; 0.00)	0.87	0.61 (-0.85; 2.07)	0.41	-0.00 (-0.01; 0.00)	0.29
	3	1.18 (-0.86; 3.22)	0.26	0.00 (-0.01; 0.01)	0.90	0.61 (-0.99; 2.22)	0.45	-0.00 (-0.01; 0.00)	0.17
ΔTotal difficulties score	1	0.32 (-0.30; 0.94)	0.31	-0.00 (-0.00; 0.00)	0.11	0.28 (-0.22; 0.79)	0.27	-0.00 (-0.00; 0.00)	0.43
	2	0.30 (-0.32; 0.92)	0.34	-0.00 (-0.00; 0.00)	0.14	0.31 (-0.20; 0.81)	0.23	-0.00 (-0.00; 0.00)	0.51
	3	0.19 (-0.55; 0.94)	0.61	-0.00 (-0.00; 0.00)	0.55	0.55 (-0.03; 1.13)	0.06	-0.00 (-0.00; 0.00)	0.25
ΔProsocial behavior	1	0.26 (-1.54; 2.07)	0.78	0.00 (-0.00; 0.01)	0.76	-0.55 (-2.00; 0.89)	0.45	0.00 (-0.00; 0.01)	0.57
	2	0.37 (-1.45; 2.19)	0.69	0.00 (-0.00; 0.01)	0.89	-0.72 (-2.16; 0.73)	0.33	0.00 (-0.00; 0.01)	0.74
	3	-0.25 (-2.34; 1.85)	0.81	0.00 (-0.01; 0.01)	0.89	-0.25 (-1.87; 1.38)	0.76	0.00 (-0.00; 0.01)	0.63

Abbreviations: CLES, Coddington Life Events Scale.
Model 1 adjusted for age, sex, socioeconomic status and baseline stress parameter
Model 2 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure
Model 3 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity

Table S5. Change in stress (emotions, negative events and behavior) over time (2012-2015) as predictor of retinal vascular diameters in 2015.

		CRAE		CRVE		AVR	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Stress							
Emotion scores							
ΔHappy	1	-0.39 (-1.71; 0.93)	0.56	**-2.37 (-4.02; -0.71)**	**0.01**	**0.01 (0.00; 0.01)**	**0.01**
	2	0.58 (-0.50; 1.66)	0.29	**-2.02 (-3.35; -0.68)**	**<0.01**	**0.01 (0.00; 0.01)**	**0.03**
	3	0.62 (-0.56; 1.79)	0.30	**-1.79 (-3.28; -0.31)**	**0.02**	**0.01 (0.00; 0.01)**	**0.05**
ΔSad	1	0.42 (-0.53; 1.37)	0.38	**2.04 (0.86; 3.22)**	**<0.001**	**-0.00 (-0.01; -0.00)**	**0.01**
	2	-0.55 (-1.32; 0.23)	0.17	**1.71 (0.77; 2.65)**	**<0.001**	**-0.00 (-0.01; -0.00)**	**0.01**
	3	-0.41 (-1.23; 0.42)	0.33	**1.59 (0.57; 2.60)**	**<0.01**	**-0.00 (-0.01; -0.00)**	**0.03**
ΔAnxious	1	0.09 (-1.11; 1.29)	0.89	0.08 (-1.45; 1.61)	0.92	0.00 (-0.00; 0.00)	0.97
	2	0.03 (-0.92; 0.98)	0.95	0.02 (-1.21; 1.25)	0.97	0.00 (-0.00; 0.00)	0.99
	3	0.26 (-0.78; 1.31)	0.62	0.26 (-1.11; 1.63)	0.71	0.00 (-0.00; 0.01)	0.90
ΔAngry	1	0.24 (-0.89; 1.36)	0.68	1.21 (-0.22; 2.64)	0.10	-0.00 (-0.01; 0.00)	0.21
	2	-0.30 (-1.20; 0.59)	0.50	1.01 (-0.13; 2.16)	0.08	-0.00 (-0.01; 0.00)	0.22
	3	-0.38 (-1.35; 0.58)	0.44	0.95 (-0.30; 2.20)	0.13	-0.00 (-0.01; 0.00)	0.24
ΔNegative emotions	1	0.14 (-0.31; 0.58)	0.54	**0.64 (0.08; 1.20)**	**0.03**	-0.00 (-0.00; 0.00)	0.09
	2	-0.16 (-0.51; 0.20)	0.39	**0.53 (0.08; 0.98)**	**0.02**	-0.00 (-0.00; 0.00)	0.09
	3	-0.11 (-0.49; 0.28)	0.58	0.50 (-0.07; 1.08)	0.08	-0.00 (-0.00; 0.00)	0.16
CLES							
ΔNegative event	1	**-0.02 (-0.04; -0.00)**	**0.02**	-0.01 (-0.03; 0.02)	0.51	-0.00 (-0.00; 0.00)	0.06
	2	**-0.02 (-0.03; -0.00)**	**0.02**	0.01 (-0.01; 0.03)	0.36	-0.00 (-0.00; 0.00)	0.07
	3	-0.01 (-0.03; 0.00)	0.10	0.00 (-0.02; 0.02)	0.64	-0.00 (-0.00; 0.00)	0.19
Behavior							
ΔConduct problems	1	0.20 (-1.31; 1.71)	0.80	1.03 (-0.92; 2.97)	0.30	-0.00 (-0.01; 0.00)	0.36
	2	-0.43 (-1.63; 0.77)	0.48	0.98 (-0.58; 2.54)	0.22	-0.00 (-0.01; 0.00)	0.24
	3	-0.38 (-1.68; 0.93)	0.57	0.45 (-1.25; 2.15)	0.60	-0.00 (-0.01; 0.00)	0.49
ΔHyperactivity problems	1	1.17 (-0.03; 2.37)	0.06	1.13 (-0.38; 2.48)	0.10	-0.00 (-0.01; 0.00)	0.65
	2	0.17 (-0.81; 1.14)	0.74	1.10 (-0.17; 2.37)	0.09	-0.00 (-0.01; 0.00)	0.49
	3	0.14 (-1.00; 1.28)	0.81	1.31 (-0.14; 2.77)	0.08	-0.00 (-0.01; 0.00)	0.43
ΔEmotional problems	1	0.37 (-0.84; 1.58)	0.55	1.69 (-0.21; 3.22)	0.09	-0.00 (-0.01; 0.00)	0.07
	2	-0.38 (-1.35; 0.60)	0.45	1.37 (-0.14; 2.60)	0.08	-0.00 (-0.01; 0.00)	0.08
	3	-0.36 (-1.43; 0.70)	0.50	1.21 (-0.14; 2.56)	0.08	-0.00 (-0.01; 0.00)	0.14
ΔPeer problems	1	-0.29 (-1.67; 1.10)	0.68	0.18 (-1.61; 1.98)	0.84	-0.00 (-0.01; 0.00)	0.46
	2	-0.41 (-1.49; 0.68)	0.46	0.44 (-0.99; 1.87)	0.54	-0.00 (-0.01; 0.00)	0.42
	3	-0.28 (-1.50; 0.93)	0.65	-0.22 (-1.81; 1.37)	0.87	-0.00 (-0.01; 0.01)	0.87
ΔProsocial behavior	1	0.96 (-0.44; 2.37)	0.18	0.44 (-1.39; 2.27)	0.64	0.00 (-0.00; 0.01)	0.27
	2	0.91 (-0.20; 2.02)	0.11	-0.44 (-11.92; 1.04)	0.56	0.00 (-0.00; 0.01)	0.17
	3	0.89 (-0.42; 2.20)	0.18	-0.36 (-2.09; 1.37)	0.68	0.00 (-0.00; 0.01)	0.25
ΔTotal difficulties score	1	0.25 (-0.23; 0.73)	0.30	0.75 (-0.14; 1.36)	0.07	-0.00 (-0.00; 0.00)	0.12
	2	-0.13 (-0.52; 0.25)	0.50	0.58 (-0.09; 1.07)	0.08	-0.00 (-0.00; 0.00)	0.08
	3	-0.12 (-0.56; 0.31)	0.58	0.48 (-0.07; 1.03)	0.09	-0.00 (-0.00; 0.00)	0.18

Abbreviations: CRAE, Central retinal arteriolar equivalent; CRVE, Central retinal venular equivalent; AVR, arteriolar to venular ratio; CLES, Coddington Life Events Scale.

Model 1 adjusted for age, sex, socioeconomic status and baseline stress parameter

Model 2 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure and alternate retinal parameter.

Model 3 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure, alternate retinal parameter, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity

Table S6. Change in stress (emotions, negative events and behavior) over time (2012-2015) as predictor of retinal network parameters in 2015.

		Arteriolar bifurcation angle		Arteriolar optimality deviation		Venular bifurcation angle		Venular optimality deviation	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Emotions									
ΔHappy	1	0.30 (-1.53; 2.13)	0.75	-0.00 (-0.01; 0.00)	0.86	0.39 (-1.09; 1.88)	0.60	-0.00 (-0.01; 0.00)	0.23
	2	0.30 (-1.55; 2.15)	0.75	-0.00 (-0.01; 0.01)	0.89	0.47 (-1.02; 1.97)	0.53	-0.00 (-0.01; 0.00)	0.30
	3	-0.03 (-2.28; 2.23)	0.98	0.00 (-0.01; 0.01)	0.93	**1.81 (0.08; 3.55)**	**0.04**	-0.00 (-0.01; 0.00)	0.12
ΔSad	1	0.12 (-1.12; 1.37)	0.85	-0.00 (-0.01; 0.00)	0.34	0.12 (-0.86; 1.09)	0.81	0.00 (-0.00; 0.00)	0.68
	2	0.12 (-1.13; 1.37)	0.85	-0.00 (-0.01; 0.00)	0.34	0.11 (-0.86; 1.09)	0.82	0.00 (-0.00; 0.00)	0.68
	3	-0.19 (-1.65; 1.27)	0.80	-0.00 (-0.00; 0.00)	0.62	0.04 (-1.05; 1.13)	0.95	0.00 (-0.00; 0.00)	0.64
ΔAnxious	1	-0.91 (-2.47; 0.64)	0.25	-0.00 (-0.00; 0.00)	0.92	0.07 (-1.16; 1.30)	0.91	-0.00 (-0.00; 0.00)	0.69
	2	-0.91 (-2.48; 0.65)	0.25	-0.00 (-0.00; 0.00)	0.93	0.08 (-1.15; 1.32)	0.89	-0.00 (-0.00; 0.00)	0.73
	3	**-2.55 (-4.57; -0.53)**	**0.01**	-0.00 (-0.01; 0.00)	0.74	-0.18 (-1.74; 1.39)	0.82	-0.00 (-0.01; 0.00)	0.06
ΔAngry	1	0.61 (-0.85; 2.08)	0.41	-0.00 (-0.01; 0.00)	0.37	0.01 (-1.14; 1.16)	0.99	0.00 (-0.00; 0.00)	0.56
	2	0.61 (-0.85; 2.08)	0.41	-0.00 (-0.01; 0.00)	0.37	-0.00 (-1.15; 1.15)	0.99	0.00 (-0.00; 0.00)	0.58
	3	0.47 (-1.28; 2.21)	0.60	-0.00 (-0.01; 0.00)	0.25	-0.64 (-1.94; 0.66)	0.33	0.00 (-0.00; 0.00)	0.64
ΔNegative emotions	1	-0.00 (-0.58; 0.58)	0.99	-0.00 (-0.00; 0.00)	0.37	0.01 (-0.44; 0.47)	0.95	0.00 (-0.00; 0.00)	0.77
	2	-0.00 (-0.59; 0.58)	0.99	-0.00 (-0.00; 0.00)	0.37	0.01 (-0.44; 0.47)	0.96	0.00 (-0.00; 0.00)	0.77
	3	-0.31 (-1.05; 0.43)	0.41	-0.00 (-0.00; 0.00)	0.40	-0.18 (-0.73; 0.37)	0.53	-0.00 (-0.00; 0.00)	0.74
CLES									
ΔNegative events	1	0.00 (-0.02; 0.03)	0.97	-0.00 (-0.00; 0.00)	0.31	-0.01 (-0.03; 0.01)	0.31	0.00 (-0.00; 0.00)	0.82
	2	0.00 (-0.02; 0.03)	0.96	-0.00 (-0.00; 0.00)	0.28	-0.01 (-0.03; 0.01)	0.28	0.00 (-0.00; 0.00)	0.90
	3	-0.00 (-0.03; 0.03)	0.91	-0.00 (-0.00; 0.00)	0.47	-0.01 (-0.03; 0.01)	0.35	0.00 (-0.00; 0.00)	0.89
Behavior									
ΔConduct problems	1	-0.29 (-2.34; 1.77)	0.78	0.00 (-0.00; 0.01)	0.75	0.70 (-0.90; 2.31)	0.39	0.00 (-0.00; 0.01)	0.18
	2	-0.33 (-2.40; 1.74)	0.75	0.00 (-0.00; 0.01)	0.65	0.81 (-0.80; 2.42)	0.32	0.00 (-0.00; 0.01)	0.12
	3	-0.17 (-2.42; 2.09)	0.88	0.00 (-0.00; 0.01)	0.24	1.28 (-0.41; 2.96)	0.14	0.00 (-0.00; 0.01)	0.07
ΔHyperactivity problems	1	-0.57 (-2.24; 1.09)	0.50	-0.00 (-0.01; 0.00)	0.28	0.82 (-0.45; 2.08)	0.20	-0.00 (-0.00; 0.00)	0.86
	2	-0.60 (-2.28; 1.08)	0.48	-0.00 (-0.01; 0.00)	0.33	0.88 (-0.39; 2.14)	0.17	-0.00 (-0.00; 0.00)	0.97
	3	-1.16 (-3.08; 0.76)	0.23	-0.00 (-0.01; 0.00)	0.18	**1.43 (0.03; 2.82)**	**0.045**	-0.00 (-0.00; 0.00)	0.90
ΔEmotional problems	1	-0.18 (-1.83; 1.48)	0.83	-0.00 (-0.01; 0.00)	0.24	0.87 (-0.39; 2.13)	0.17	0.00 (-0.00; 0.01)	0.49
	2	-0.16 (-1.82; 1.50)	0.85	-0.00 (-0.01; 0.00)	0.22	0.84 (-0.42; 2.10)	0.19	0.00 (-0.00; 0.01)	0.53

	3	-0.47 (-2.28; 1.34)	0.61	-0.00 (-0.01; 0.00)	0.12	1.29 (-0.03; 2.62)	0.06	0.00 (-0.00; 0.01)	0.54
ΔPeer problems	1	1.12 (-0.83; 3.07)	0.26	0.00 (-0.01; 0.01)	0.91	0.67 (-0.83; 2.18)	0.38	-0.00 (-0.01; 0.00)	0.96
	2	1.11 (-0.84; 3.07)	0.26	0.00 (-0.01; 0.01)	0.88	0.68 (-0.83; 2.18)	0.37	-0.00 (-0.00; 0.00)	0.97
	3	0.94 (-1.24; 3.12)	0.39	0.00 (-0.00; 0.01)	0.73	0.65 (-0.96; 2.27)	0.42	-0.00 (-0.01; 0.00)	0.78
ΔTotal difficulties score	1	-0.08 (-0.76; 0.59)	0.81	-0.00 (-0.00; 0.00)	0.44	0.45 (-0.07; 0.97)	0.09	0.00 (-0.00; 0.00)	0.54
	2	-0.09 (-0.77; 0.59)	0.79	-0.00 (-0.00; 0.00)	0.50	0.47 (-0.05; 0.99)	0.07	0.00 (-0.00; 0.00)	0.46
	3	-0.31 (-1.07; 0.45)	0.42	-0.00 (-0.00; 0.00)	0.51	**0.65 (0.09; 1.21)**	**0.02**	0.00 (-0.00; 0.00)	0.53
ΔProsocial behavior	1	-0.29 (-2.19; 1.62)	0.77	-0.00 (-0.01; 0.00)	0.54	-1.13 (-2.56; 0.30)	0.12	0.00 (-0.00; 0.01)	0.74
	2	-0.24 (-2.16; 1.68)	0.80	-0.00 (-0.01; 0.00)	0.47	-1.25 (-2.68; 0.19)	0.09	0.00 (-0.00; 0.01)	0.90
	3	-1.11 (-3.64; 1.15)	0.33	-0.00 (-0.01; 0.00)	0.60	-0.46 (-2.11; 1.19)	0.58	0.00 (-0.00; 0.01)	0.68

Abbreviations: CLES, Coddington Life Events Scale.
Model 1 adjusted for age, sex, socioeconomic status and baseline stress parameter
Model 2 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure
Model 3 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity

Table S7. Change in stress (emotions, negative events and behavior) over time (2013-2015) as predictor of retinal vascular diameters in 2015.

		CRAE		CRVE		AVR	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Stress							
Emotion scores							
ΔHappy	1	0.00 (-1.40; 1.41)	0.99	**-1.93 (-3.61; -0.25)**	**0.02**	**0.01 (0.00; 0.01)**	**0.01**
	2	0.82 (-0.31; 1.96)	0.15	**-1.80 (-3.15; -0.46)**	**0.01**	**0.01 (0.00; 0.01)**	**0.02**
	3	0.80 (-0.44; 2.03)	0.21	**-1.63 (-3.15; -0.11)**	**0.04**	**0.01 (0.00; 0.01)**	**0.05**
ΔSad	1	0.17 (-0.93; 1.26)	0.76	**1.54 (0.242; 2.83)**	**0.02**	**-0.00 (-0.01; -0.00)**	**0.05**
	2	-0.60 (-1.48; 0.27)	0.18	**1.39 (0.37; 2.42)**	**0.01**	**-0.00 (-0.01; -0.00)**	**0.05**
	3	-0.47 (-1.42; 0.49)	0.34	**1.20 (0.05; 2.35)**	**0.04**	-0.00 (-0.01; 0.00)	0.13
ΔAnxious	1	-0.43 (-1.77; 0.91)	0.53	0.266 (-1.36; 1.89)	0.75	-0.00 (-0.01; 0.00)	0.26
	2	-0.51 (-1.57; 0.55)	0.34	0.55 (-0.76; 1.85)	0.41	-0.00 (-0.01; 0.00)	0.30
	3	-0.28 (-1.47; 0.91)	0.64	0.55 (-0.94; 2.04)	0.47	-0.00 (-0.01; 0.00)	0.53
ΔAngry	1	0.53 (-0.66; 1.73)	0.38	0.99 (-0.46; 2.43)	0.18	-0.00 (-0.01; 0.00)	0.76
	2	0.08 (-0.88; 1.04)	0.87	0.57 (-0.60; 1.74)	0.34	-0.00 (-0.01; 0.00)	0.80
	3	0.06 (-0.98; 1.11)	0.91	0.64 (-0.67; 1.94)	0.34	-0.00 (-0.01; 0.00)	0.75
ΔNegative emotions	1	0.04 (-0.45; 0.53)	0.87	0.51 (-0.09; 1.10)	0.09	-0.00 (-0.00; 0.00)	0.13
	2	-0.20 (-0.59; 0.20)	0.32	0.46 (-0.01; 0.94)	0.06	-0.00 (-0.00; 0.00)	0.14
	3	-0.14 (-0.57; 0.29)	0.52	0.44 (-0.09; 0.97)	0.10	-0.00 (-0.00; 0.00)	0.25
CLES							
ΔNegative event	1	**-0.02 (-0.03; -0.00)**	**0.04**	-0.00 (-0.02; 0.02)	0.78	**-0.00 (-0.00; -0.00)**	**0.05**
	2	**-0.02 (-0.03; -0.00)**	**0.01**	0.01 (-0.01; 0.03)	0.20	**-0.00 (-0.00; -0.00)**	**0.04**
	3	**-0.02 (-0.03; -0.00)**	**0.03**	0.01 (-0.01; 0.03)	0.20	-0.00 (-0.00; 0.00)	0.06
Behavior							
ΔConduct problems	1	-0.24 (-1.94; 1.46)	0.78	-0.14 (-2.28; 2.00)	0.90	-0.00 (-0.01; 0.01)	0.82
	2	-0.25 (-1.64; 1.14)	0.73	0.09 (-1.68; 1.86)	0.92	-0.00 (-0.01; 0.01)	0.75
	3	-0.03 (-1.65; 1.58)	0.97	-0.53 (-2.52; 1.47)	0.60	0.00 (-0.01; 0.01)	0.86
ΔHyperactivity problems	1	-0.05 (-1.28; 1.17)	0.93	-0.58 (-2.12; 0.96)	0.46	0.00 (-0.00; 0.01)	0.52
	2	0.14 (-0.86; 1.13)	0.79	-0.47 (-1.74; 0.79)	0.46	0.00 (-0.00; 0.01)	0.62
	3	0.02 (-1.14; 1.19)	0.97	-0.36 (-1.79; 1.07)	0.62	0.00 (-0.00; 0.01)	0.80
ΔEmotional problems	1	-0.05 (-1.37; 1.27)	0.94	0.18 (-1.49; 1.85)	0.83	-0.00 (-0.01; 0.00)	0.69
	2	-0.13 (-1.21; 0.95)	0.82	0.21 (-1.16; 1.59)	0.76	-0.00 (-0.01; 0.00)	0.69
	3	-0.16 (-1.38; 1.05)	0.79	-0.14 (-1.64; 1.36)	0.85	-0.00 (-0.01; 0.01)	0.87
ΔPeer problems	1	-0.23 (-1.70; 1.24)	0.76	-0.45 (-2.30; 1.40)	0.63	0.00 (-0.01; 0.01)	0.86
	2	-0.04 (-1.24; 1.16)	0.95	-0.27 (-1.80; 1.25)	0.72	0.00 (-0.01; 0.01)	0.87
	3	0.27 (-1.14; 1.67)	0.71	-1.06 (-2.76; 0.65)	0.22	0.00 (-0.00; 0.01)	0.39
ΔProsocial behavior	1	0.54 (-0.78; 1.86)	0.42	0.16 (-1.50; 1.82)	0.85	0.00 (-0.00; 0.01)	0.43
	2	0.51 (-0.57; 1.58)	0.36	-0.26 (-1.64; 1.11)	0.70	0.00 (-0.00; 0.01)	0.38
	3	0.38 (-0.87; 1.64)	0.55	0.13 (-1.42; 1.69)	0.87	0.00 (-0.00; 0.01)	0.68
ΔTotal difficulties score	1	-0.04 (-0.57; 0.50)	0.89	-0.14 (-0.82; 0.54)	0.69	0.00 (-0.00; 0.00)	0.83
	2	0.01 (-0.43; 0.44)	0.98	-0.09 (-0.65; 0.46)	0.74	0.00 (-0.00; 0.00)	0.90
	3	0.06 (-0.45; 0.57)	0.81	-0.34 (-0.97; 0.28)	0.28	0.00 (-0.00; 0.00)	0.53

Abbreviations: CRAE, Central retinal arteriolar equivalent; CRVE, Central retinal venular equivalent; AVR, arteriolar to venular ratio; CLES, Coddington Life Events Scale.

Model 1 adjusted for age, sex, socioeconomic status and baseline stress parameter

Model 2 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure and alternate retinal parameter.

Model 3 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure, alternate retinal parameter, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity

Table S8. Change in stress (emotions, negative events and behavior) over time (2013-2015) as predictor of retinal network parameters in 2015.

		Arteriolar bifurcation angle		Arteriolar optimality deviation		Venular bifurcation angle		Venular optimality deviation	
	Model	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*	*B (95%CI)*	*P-value*
Emotions									
ΔHappy	1	0.71 (-1.23; 2.65)	0.47	-0.01 (-0.01; 0.00)	0.07	0.72 (-0.88; 2.31)	0.38	**-0.01 (-0.01; -0.00)**	**0.048**
	2	0.74 (-1.21; 2.70)	0.45	-0.01 (-0.01; 0.00)	0.07	0.79 (-0.82; 2.40)	0.33	-0.00 (-0.01; 0.00)	0.07
	3	0.63 (-1.72; 2.97)	0.60	-0.00 (-0.01; 0.00)	0.27	1.73 (-0.07; 3.54)	0.06	-0.01 (-0.01; 0.00)	0.06
ΔSad	1	0.18 (-1.14; 1.50)	0.79	-0.00 (-0.00; 0.00)	0.95	-0.12 (-1.23; 0.99)	0.83	0.00 (-0.00; 0.00)	0.78
	2	0.18 (-1.14; 1.51)	0.79	-0.00 (-0.00; 0.00)	0.95	-0.12 (-1.23; 0.99)	0.83	0.00 (-0.00; 0.00)	0.78
	3	-0.09 (-1.66; 1.48)	0.91	-0.00 (-0.01; 0.00)	0.77	-0.45 (-1.71; 0.80)	0.47	0.00 (-0.00; 0.00)	0.95
ΔAnxious	1	-0.65 (-2.34; 1.04)	0.45	0.00 (-0.00; 0.01)	0.74	-0.39 (-1.78; 0.99)	0.57	0.00 (-0.00; 0.00)	0.83
	2	-0.65 (-2.35; 1.04)	0.45	0.00 (-0.00; 0.01)	0.74	-0.40 (-1.78; 0.98)	0.57	0.00 (-0.00; 0.00)	0.84
	3	-1.82 (-4.19; 0.54)	0.13	-0.00 (-0.01; 0.01)	0.99	-1.01 (-2.86; 0.84)	0.28	-0.00 (-0.01; 0.00)	0.62
ΔAngry	1	0.46 (-1.01; 1.93)	0.54	0.00 (-0.00; 0.01)	0.79	-0.26 (-1.47; 0.95)	0.68	0.00 (-0.00; 0.00)	0.67
	2	0.46 (-1.02; 1.93)	0.54	0.00 (-0.00; 0.01)	0.81	-0.27 (-1.48; 0.95)	0.66	0.00 (-0.00; 0.00)	0.70
	3	0.90 (-0.88; 2.68)	0.32	-0.00 (-0.01; 0.00)	0.79	-0.75 (-2.16; 0.66)	0.30	0.00 (-0.00; 0.01)	0.70
ΔNegative emotions	1	0.04 (-0.57; 0.65)	0.90	0.00 (-0.00; 0.00)	0.76	-0.14 (-0.65; 0.36)	0.57	0.00 (-0.00; 0.00)	0.75
	2	0.04 (-0.57; 0.65)	0.90	0.00 (-0.00; 0.00)	0.76	-0.15 (-0.65; 0.36)	0.56	0.00 (-0.00; 0.00)	0.77
	3	-0.07 (-0.84; 0.70)	0.86	-0.00 (-0.00; 0.00)	0.85	-0.37 (-0.99; 0.24)	0.23	-0.00 (-0.00; 0.00)	0.96
CLES									
ΔNegative events	1	-0.01 (-0.03; 0.01)	0.66	-0.00 (-0.00; 0.00)	0.08	-0.01 (-0.03; 0.01)	0.29	0.00 (-0.00; 0.00)	0.90
	2	-0.01 (-0.03; 0.02)	0.66	-0.00 (-0.00; 0.00)	0.08	-0.01 (-0.03; 0.01)	0.29	0.00 (-0.00; 0.00)	0.88
	3	-0.01 (-0.03; 0.02)	0.57	-0.00 (-0.00; 0.00)	0.16	-0.01 (-0.03; 0.01)	0.32	0.00 (-0.00; 0.00)	0.88
SDQ									
ΔConduct problems	1	-0.57 (-2.97; 1.83)	0.64	0.00 (-0.00; 0.01)	0.36	0.08 (-1.73; 1.89)	0.93	0.00 (-0.00; 0.01)	0.30
	2	-0.56 (-2.97; 1.85)	0.65	0.00 (-0.00; 0.01)	0.35	0.09 (-1.72; 1.91)	0.92	0.00 (-0.00; 0.01)	0.26
	3	-0.98 (-3.78; 1.82)	0.49	0.01 (-0.00; 0.01)	0.09	-0.40 (-2.40; 1.60)	0.69	0.00 (-0.00; 0.01)	0.48
ΔHyperactivity problems	1	0.09 (-1.63; 1.81)	0.92	-0.00 (-0.01; 0.00)	0.54	0.32 (-0.95; 1.59)	0.62	-0.00 (-0.01; 0.00)	0.45
	2	0.09 (-1.64; 1.82)	0.91	-0.00 (-0.01; 0.00)	0.56	0.34 (-0.94; 1.61)	0.60	-0.00 (-0.01; 0.00)	0.52
	3	0.19 (-1.81; 2.19)	0.85	-0.00 (-0.01; 0.01)	0.97	0.49 (-0.92; 1.91)	0.49	-0.00 (-0.00; 0.00)	0.91
ΔEmotional problems	1	-0.05 (-1.97; 1.87)	0.96	0.00 (-0.01; 0.01)	0.96	0.92 (-0.47; 2.32)	0.19	0.00 (-0.00; 0.01)	0.28
	2	-0.05 (-1.98; 1.88)	0.96	0.00 (-0.01; 0.01)	0.94	0.94 (-0.46; 2.33)	0.19	0.00 (-0.00; 0.01)	0.25

	3	0.07 (-2.10; 2.25)	0.95	0.00 (-0.00; 0.01)	0.77	1.24 (-0.27; 2.76)	0.11	0.00 (-0.00; 0.01)	0.24
ΔPeer problems	1	1.26 (-0.86; 3.38)	0.24	-0.00 (-0.01; 0.01)	0.98	-0.09 (-1.70; 1.52)	0.91	-0.00 (-0.01; 0.00)	0.50
	2	1.26 (-0.86; 3.89)	0.25	-0.00 (-0.01; 0.01)	0.98	-0.09 (-1.71; 1.52)	0.92	-0.00 (-0.01; 0.00)	0.49
	3	1.59 (-0.89; 4.07)	0.21	0.00 (-0.00; 0.01)	0.48	-0.41 (-2.18; 1.37)	0.65	-0.00 (-0.01; 0.00)	0.22
ΔTotal difficulties score	1	0.13 (-0.67; 0.92)	0.75	0.00 (-0.00; 0.00)	0.93	0.24 (-0.35; 0.83)	0.42	0.00 (-0.00; 0.00)	0.85
	2	0.13 (-0.67; 0.93)	0.75	0.00 (-0.00; 0.00)	0.90	0.24 (-0.35; 0.84)	0.42	0.00 (-0.00; 0.00)	0.78
	3	0.19 (-0.75; 1.13)	0.69	0.00 (-0.00; 0.00)	0.32	0.23 (-0.44; 0.89)	0.50	0.00 (-0.00; 0.00)	0.90
ΔProsocial behavior	1	-0.06 (-1.90; 1.78)	0.95	0.00 (-0.00; 0.01)	0.45	-1.29 (-2.63; 0.04)	0.06	0.00 (-0.00; 0.01)	0.56
	2	-0.06 (-1.91; 1.79)	0.95	0.00 (-0.00; 0.01)	0.46	-1.31 (-2.65; 0.03)	0.06	0.00 (-0.00; 0.01)	0.61
	3	-0.99 (-3.15; 1.18)	0.37	0.00 (-0.00; 0.01)	0.66	-0.31 (-1.82; 1.21)	0.69	0.00 (-0.00; 0.01)	0.20

Model 1 adjusted for age, sex, socioeconomic status and baseline stress parameter
Model 2 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure
Model 3 adjusted for age, sex, socioeconomic status, baseline stress parameter, mean arterial pressure, zBMI, sugar propensity ratio, fat propensity ratio, sleep duration and physical activity