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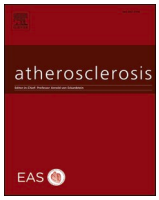

Associations of long-term cumulative C-reactive protein and glycoprotein acetyls concentrations in childhood, adolescence and adulthood with adulthood retinal microvascular structure

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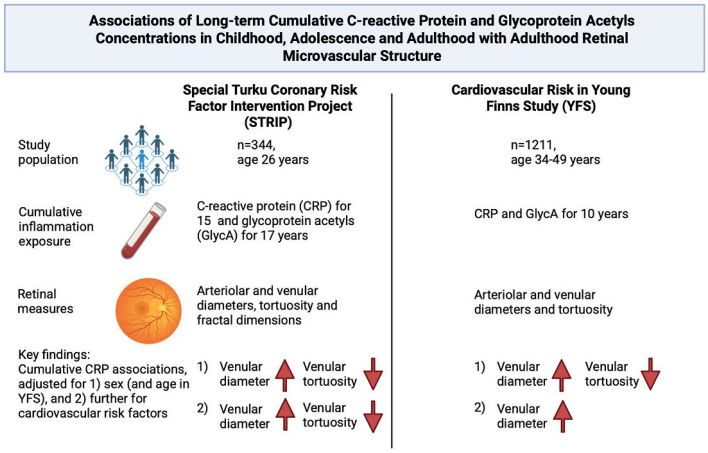
HIGHLIGHTS

- Long-term cumulative *C*-reactive protein exposure was associated with wider retinal venules in two population based-cohorts.
- The association between cumulative *C*-reactive protein and venular tortuosity differed between young and mid-adulthood.
- Cumulative glycoprotein acetyls was not associated with retinal microvascular measures.

GRAPHICAL ABSTRACT



ABSTRACT

Backgroung and aims: Inflammation is associated with cardiovascular disease development and microvascular dysfunction. The aim of the present study is to test the hypothesis that long-term exposure to chronic inflammation in childhood and adulthood is associated with adverse retinal microvascular structure in young and mid-adulthood.

Methods: We analyzed data derived from the Special Turku Coronary Risk Factor Intervention Project (STRIP) and longitudinal Cardiovascular Risk in Young Finns Study (YFS). In STRIP, fundus photos were taken in young adulthood (aged 26 years), and in YFS in mid-adulthood (aged 34–49 years). Retinal microvascular measures were derived in both cohorts (arteriolar and venular diameters and tortuosity; additionally, fractal dimensions in STRIP). Cumulative exposure as the area under the curve for high-sensitivity *C*-reactive protein (hsCRP) and glycoprotein acetyls (GlycA), and other conventional cardiovascular risk factors was determined over a 15- and 17-year period in STRIP, and a 10-year period in YFS. Overall, retinal microvascular and cumulative hsCRP and/or GlycA were available for 344 STRIP and 1211 YFS participants, thus forming the cohort of the present study.

Results: In both cohorts, cumulative hsCRP was associated with wider venules when adjusted for sex (and age in YFS), and further for related cardiovascular risk factors. In young adulthood (STRIP), higher exposure to cumulative hsCRP was associated with decreased venular tortuosity, whereas in mid-adulthood (YFS), the association was inverse. Cumulative hsCRP was not associated with arteriolar measures whereas cumulative GlycA showed no significant association with any retinal microvascular measures.

Conclusions: Long-term cumulative hsCRP exposure was associated with wider venules in young and mid-adulthood, whereas the associations with venular tortuosity were inconsistent. Wider retinal venules might act as a marker for cumulative inflammatory burden.

1. Introduction

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality globally [1]. It has been previously hypothesized that adverse microvascular changes play a role in a wide range of cardiovascular damage evolution [2,3]. In addition, inflammation seems to contribute to both CVD [4], and especially coronary and peripheral microvascular dysfunction development [2,3], and coronary microvascular dysfunction is repeatedly associated with elevated levels of inflammatory markers [5]. In line, chronic inflammatory rheumatoid diseases are associated with coronary microvascular dysfunction, suggesting that prolonged systemic inflammation may contribute to microvascular dysfunction and cardiovascular disease development [6,7].

The microvascular dysfunction of one organ seems to reflect systemic microvascular dysfunction [8], and therefore imaging of the retinal microvasculature offers a unique, non-invasive approach to assess systemic microvasculature. Indeed, retina has previously been described as window to the heart [9], and retinal microvascular derived parameters, such as vessel diameters, fractal dimension and tortuosity, are predictive of cardiovascular morbidity and mortality [10–13].

Previous retinal studies, which have primarily investigated associations with *C*-reactive protein (CRP) and white blood cell count, have associated inflammatory marker levels with wider and more tortuous retinal venules [14,15]. These results, however, are from cross-sectional studies, and studies on associations of long-term inflammation with retinal microvasculature are lacking. In addition, to our knowledge, studies on associations of long-term cumulative inflammatory marker levels with any microvascular bed are lacking.

Utilizing data from two longitudinal cohorts, Special Turku Coronary Risk Factor Intervention Project (STRIP) [16] and Cardiovascular risk in Young Finns Study (YFS) [17], we were able to study associations of long-term (up to 17 and 10 years, respectively) cumulative levels of inflammatory markers (high-sensitivity CRP [hsCRP] and a novel inflammation marker Glycoprotein acetyls [18] [GlycA]) with several retinal microvasculature-derived measures in young and mid-adulthood. GlycA is a nuclear magnetic resonance–derived biomarker that has been reported to predict cardiovascular morbidity and mortality [19]. It has greater long-term stability than CRP and may reflect distinct aspects of the inflammatory response [20]. Therefore, combining GlycA with CRP may provide complementary insight into early inflammation-related vascular changes. Additionally, utilizing data from YFS enabled us to study the associations of a single childhood hsCRP measure with mid-adulthood retinal microvasculature after over 30 years of follow-up. The aim of the present study was to test the hypothesis that long-term cumulative chronic inflammation is associated with adverse retinal microvascular structure.

2. Methods

2.1. The Special Turku Coronary Risk Factor Intervention Project (STRIP)

The STRIP study is a randomized, prospective intervention trial aiming to reduce the exposure to known environmental atherosclerosis risk factors from infancy to early adulthood. The study design, participant recruitment, dietary intervention, and data collection methods have been detailed previously [16]. Briefly, a total of 45 children born between March and July of 1989 were recruited and randomized (intervention n = 22, control n = 23, females n = 26) to test the study protocols and serve as a pilot group. Later, families of healthy 5-month--old infants, born between July 1989 and December 1991, were recruited by nurses during routine visits to well-baby clinics in the city of Turku, Finland. At the age of 7 months, 1062 infants (56.2 % of the eligible age cohort) and their families commenced the study and were randomized to either the dietary intervention (n = 540; 256 girls) or control (n = 522; 256 girls) group. Both the intervention and control groups attended regular clinic visits that were led by a pediatrician or a study nurse and a dietitian.

The STRIP intervention group had study visits at 1- to 3-month intervals until the child was aged 2 years, whereas the control group had study visits biannually. After the age of 2 years, children in both groups had biannual visits, and when the control group reached age 7 years, they had annual visits. Study visits continued until participants reached the age of 20 years.

The first post-intervention follow-up study was conducted between April 2015 and January 2018 at the age of 26 years, six years after the intervention had ended [21]. Of the study participants, 551 provided follow-up data (51 %; intervention, n = 263 vs. control, n = 288), and five of them provided only questionnaire data. Loss-to-follow-up at age 26 years and reasons for non-participation has been previously reported; briefly, those who have stayed in the study have been similar to those who withdrew [21].

2.2. The cardiovascular risk in Young Finns Study (YFS)

YFS is an ongoing prospective multicenter study from Finland initiated in the late 1970s [17]. The study has been carried out in all five Finnish university cities with medical schools and their rural surroundings. The first cross-sectional study was conducted in 1980. Altogether, 4320 children and adolescents aged 3, 6, 9, 12, 15 and 18 years were randomly chosen from the population register of these areas to produce a representative sample of Finnish children. The whole cohort has been followed in regular intervals in 1983, 1986, 1989, 2001, 2007 and 2011. Detailed description on the population and protocols has been previously reported [17]. Loss to follow-up has also been reported previously [22]; briefly, when compared to non-participants, participants were more often females and older than non-participants with no significant differences in modifiable cardiovascular risk factors at baseline.

2.3. Retinal measurements

In both STRIP and YFS, 45° digital retinal imaging was performed using a Canon nonmydriatic retinal camera (Canon CR6-45NM) fitted with a Canon 10D digital SLR camera (resolution: 3072 × 2048 pixels). Disc-centered and macula-centered images were obtained from all participants. In YFS, retinal imaging was performed in the year 2011 follow-up study (participants aged 34–49 years), and in STRIP during the 26-year follow-up visit between the years 2015 and 2018. Different methods were utilized to extract retinal microvascular measurements between the study cohorts.

In STRIP, disc-centered retinal images were analyzed by trained graders using a semi-automated computer-based program (Singapore I Vessel Assessment [SIVA], version 4, National University of Singapore, Singapore) [23,24]. These trained graders were blinded to participant data. Retinal vascular measures were assessed quantitatively at 0.5–2.0 disc diameters (zone C) from the optic disc margin. The inter- and intra-individual variability of the used method has been previously reported [24]. Details of the SIVA method utilized in the STRIP study have been previously reported [25].

The analyzed retina-derived variables included the following:

- Arteriolar and venular diameters using six widest arterioles and venules summarized as central retinal arteriole equivalent (CRAE) and central retinal venule equivalent (CRVE)
- Arteriolar and venular fractal dimensions calculated from a skeletonized line tracing using the box-counting method and representing a “global” measure summarizing the entire branching pattern of the retinal vasculature, with larger values representing a more complex pattern
- Arteriolar and venular simple tortuosity estimated as the actual path length of the vessel segment divided by the straight-line length
- Arteriolar and venular curvature tortuosity derived from the integral of the curvature square along the path of the vessel, normalized by the total path length.

In YFS, A semi-automated grading system was used to capture a range of vascular geometric parameters from macula-centered fundus photos including [26]:

- Arteriolar and venular diameters
- Arteriolar and venular tortuosity; estimated as the actual length of the vessel divided by the straight-line distance between bifurcations, minus one

In this study, 1 pixel corresponds to approximately 5 μm of the estimated diameter of arterioles (~100 μm). The arteriolar and venular diameters were measured at a series of cross-sections normal to the vessel at 2-pixel intervals along the entire length of the vessel segment. At each cross-section, the vessel diameter was measured to sub-pixel accuracy using a sliding linear regression filter technique as described previously and an average calculated for each vessel. [27]. Details of the method and reproducibility of this technique has been reported previously [26–29]. In the present study reproducibility of this technique was excellent (intra-class correlation coefficients for within-observer measurements were >0.9) and the average absolute difference and standard deviation (SD) between measurements of arteriolar diameter was 0.0 ± 0.4 pixels consistent with previous reports.

For interpretability, the retinal measures of the STRIP and YFS studies were standardized per study resulting in variables with a mean 0 and SD 1.

2.4. Inflammatory markers

In both studies, serum hsCRP was measured by automated analyzer (Olympus AU400) using a turbidimetric immunoassay kit (“CRP-UL”-assay, Wako Chemicals, Neuss, Germany). GlycA in both studies was measured as part of a nuclear magnetic resonance (NMR) metabolomics platform (Nightingale Health, Helsinki, Finland) as described elsewhere [30,31]. GlycA is an integral part of the automated molecular output of the NMR metabolomics platform and is quantified based on its molecule-specific resonance in the NMR spectrum. GlycA is considered a marker of chronic inflammation and reflects a combined measure of several glycoproteins circulating in the blood – primarily alpha-1 antitrypsin, alpha-1-acid glycoprotein, haptoglobin, transferrin, and alpha-1-antichymotrypsin – which respond to a wide range of inflammatory stimuli [32,33].

In STRIP, GlycA was measured at the age of 9 years, and thereafter GlycA and hsCRP were measured during follow-ups at ages 11, 13, 15, 17, 19 and 26 (with additional hsCRP measurements at ages 18 and 20).

In YFS, hsCRP and GlycA were measured during follow-ups in 2001, 2007 and 2011. Additionally, childhood hsCRP levels from serum samples taken in 1980 was analyzed in 2005 [34].

To assess long-term low-grade inflammation, hsCRP values greater than 10 mg/L indicating acute inflammation were excluded. GlycA has previously been shown to be considerably more stable over time than CRP and has been hypothesized to be representative of longer-term exposure to cumulative inflammatory burden [20], and therefore GlycA values were utilized as such.

2.5. Other cardiovascular risk factors

In both studies, standard methods were used for measuring blood pressure, serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, as well as glucose and insulin concentrations. The details of these methods have been described previously [17,21]. All laboratory measurements utilized in this study were from fasting serum samples. Low-density lipoprotein (LDL) cholesterol was calculated according to Friedewald [35]. Weight and height were measured, and body mass index (BMI) was calculated.

In STRIP, blood pressure, total cholesterol, and HDL cholesterol were measured at baseline and annually thereafter until age 20 years. Triglycerides were measured since the age of 5 years, and fasting serum glucose and insulin were measured annually from age 7 to 20. At all annual visits, the participants' weight and height were measured and BMI was calculated. These measurements were repeated at the first post-intervention follow-up at age 26.

In YFS, data were collected at baseline (1980) and at all follow-up studies (except for serum glucose, which was measured since the 1986 follow-up).

Data on smoking habits were collected via questionnaires. In both studies, regular smoking was defined as smoking at least once per day at the time of the retinal assessment. Additionally, in YFS, data on daily smoking in 1980 was collected via questionnaires from the participants aged 12 years and older.

2.6. Cumulative hsCRP, GlycA and other cardiovascular risk factors

To assess cumulative exposure to hsCRP and GlycA in both the STRIP and the YFS cohorts, we used linear interpolation to construct an area under the curve (AUC) variable. Similarly, we constructed an AUC variable for cumulative systolic blood pressure, BMI, LDL-cholesterol, HDL-cholesterol, triglycerides and glucose over the related time ranges. The approach is similar to Huang et al. [36] In YFS, hsCRP and GlycA AUCs were constructed for a 10-year period utilizing measures from the adulthood follow-ups (years 2001, 2007 and 2011). In STRIP, CRP AUC was constructed for a maximum of 15-year period (between ages 11–26 years) and for GlycA a maximum of 17-year period (between ages 9–26 years).

In STRIP, hsCRP and GlycA AUCs were constructed for participants with cross-sectional and a minimum of three additional hsCRP or GlycA measurements, respectively, available. In YFS, hsCRP and GlycA AUCs were constructed for participants with hsCRP or GlycA measurements, respectively, available from all three adulthood follow-ups.

To control for differences in the longest available AUC durations in the STRIP study, absolute values of the AUCs were divided by the available follow-up time, thus offering long-term average annual exposure. To make AUC values comparable between the STRIP and YFS cohorts, AUCs were similarly time-averaged in YFS.

2.6.1. Participants of the present study

STRIP: In total, 546 participants attended the STRIP 26-year follow-up study visit, of whom 486 had retinal data available for the analyses. The flow-chart of the STRIP study at the time of retinal assessment has been published previously [25]. Of these participants, 484 and 483 participants provided the required hsCRP and GlycA measurements, respectively, at the time of retinal assessment. Of those, 16 participants had hsCRP greater than 10 mg/L at the 26-year follow-up, and thus were excluded from constructing the hsCRP AUC. After further exclusion of participants with less than four hsCRP (hsCRP less than 10 mg/L) or GlycA measurements available, cumulative hsCRP and GlycA AUCs were constructed for 326 and 343 participants, respectively. Overall, 344 participants provided either the required hsCRP or GlycA data.

YFS: In total, 2063 participants attended the YFS follow-up study visit in the year 2011 (participants aged 34–49 years), of whom 1684 had retinal data available for the analyses. Of these participants, 102 were excluded from the formation of the cumulative hsCRP AUC due to the hsCRP level greater than 10 mg/L at one or more time points. In total, required hsCRP and GlycA data from all three time points (years 2001, 2007 and 2011) to construct AUCs was available for 1131 and 1162 participants, respectively. Data on the required hsCRP or GlycA was provided by 1211 participants. Both studies were approved by local ethics committees and all participants provided written informed consent.

2.7. Statistical analysis

All variables were checked for normality. The normality of the variable distributions was assessed visually by examining the shapes of the distributions and variables with right-skew distribution were transformed using natural logarithmic transformation (arteriolar tortuosity [YFS], curvature venular and arteriolar tortuosity [STRIP], cumulative hsCRP, triglycerides, insulin and glucose. Additionally, YFS year 1980 hsCRP, insulin and triglycerides).

Associations of cumulative hsCRP and GlycA with the standardized (mean 0, SD 1) retinal microvascular outcome measures were studied using linear regression models. Primarily, in both STRIP and YFS the models were fitted with sex as a covariate. Simultaneously, in STRIP models studying associations with arteriolar curvature tortuosity and fractal dimension and venular curvature tortuosity were adjusted for the STRIP intervention study group due to the previously reported dietary counselling intervention effect on these retinal measures [25]. In YFS all models were adjusted also for age. In STRIP, all participants were 26 years old at the time of the post-intervention follow-up, and therefore models were not adjusted for age. Secondly, models showing an association on the conventional statistical significance level ($p < 0.05$) between cumulative hsCRP or GlycA and retinal measures were selected for further adjustments for confounding cardiovascular risk factors. To control for wide range of confounding cardiovascular risk factors, each cumulative cardiovascular risk factor and smoking status showing a sex (and in YFS age) adjusted association $p < 0.1$ with a given retinal variable was included in the related model. This approach to confounder selection is consistent with our previous publication [25]. In STRIP, associations of cumulative cardiovascular risk factors with retinal measures have been previously reported [25], and these associations were utilized in confounder selection. Associations of cumulative cardiovascular risk factors in YFS and smoking status in STRIP and YFS with retinal measures are shown in supplemental material and were utilized in confounder selection (Supplemental Tables 1 and 2). These per study and retinal variable selected confounders are later referred to as "related cardiovascular risk factors".

Possible effect modification caused by sex on the associations between cumulative hsCRP and GlycA and the retinal variables was tested. Additionally, in YFS possible effect modification caused by age group at the time of retinal assessment on the associations between cumulative hsCRP and GlycA and the retinal variables was tested.

Furthermore, in YFS associations of the 31-year prior baseline (year 1980) childhood/adolescence hsCRP measure with retinal measures in mid-adulthood were studied using linear models. Similarly, models were primarily adjusted for age and sex, and models showing an association ($p < 0.1$) further for related cardiovascular risk factors levels in the year 1980 (associations are shown in the Supplemental Table 3).

The data analyses were performed with SAS 9.4 (SAS Institute, Inc., Cary, North Carolina).

3. Results

Table 1 describes characteristics of the study population. Characteristics of participants with cumulative CRP or GlycA data available are presented separately in Supplemental Table 4. Characteristics of the retinal measures, and cumulative hsCRP and GlycA AUCs are shown in Supplemental Tables 5 and 6 Furthermore, cross-sectional associations of hsCRP and GlycA with retinal measures in young and mid-adulthood are shown in Supplemental Tables 7 and 8 Correlations between hsCRP and GlycA AUCs are shown in Supplemental Table 9.

In young adulthood (STRIP), higher cumulative hsCRP was associated with increased venular diameter and decreased venular curvature and simple tortuosity when adjusted for sex (and models on venular curvature tortuosity and arteriolar fractal dimension for STRIP intervention study group) (Table 2). When models studying associations between hsCRP and venular diameter, and simple and curvature tortuosity were further adjusted for related cardiovascular risk factors (models adjusted for per retinal variable: smoking, systolic blood pressure and triglycerides; triglycerides; and insulin, respectively), hsCRP remained associated with decreased venular simple and curvature tortuosity, while the association with venular diameter slightly weakened and failed to meet the threshold for conventional statistical significance. GlycA was not significantly associated with retinal variables at the conventional significance threshold.

In mid-adulthood (YFS), higher cumulative hsCRP was associated with decreased arteriolar diameter, and increased venular diameter and tortuosity when adjusted for age and sex (Table 3). When the analyses were further adjusted for related cardiovascular risk factors (models adjusted for per retinal variable: arteriolar diameter: systolic blood pressure, BMI, LDL-cholesterol, triglycerides and insulin; venular diameter: smoking; and venular tortuosity: systolic blood pressure, BMI, glucose and triglycerides), cumulative hsCRP was still associated with increased venular diameter, whereas the other associations attenuated. GlycA was not significantly associated with retinal variables at the conventional significance threshold.

Additionally, in YFS, 31-year prior hsCRP measure was associated with increased venular tortuosity in mid-adulthood when adjusted for age and sex, and further for related cardiovascular risk factors (systolic blood pressure) (Table 4). No other associations between the 1980 hsCRP and retinal measures were detected.

There was no effect modification caused by sex on associations between cumulative hsCRP or GlycA with retinal variables in young (STRIP) or mid-adulthood (YFS) (data not shown). Additionally, no effect modification caused by age at the time of the retinal assessment in YFS (participants aged 34, 37, 40, 43, 46 or 49 years) was detected (data not shown).

Table 1
Characteristics of the Special Turku Coronary Risk Factor Intervention Project (STRIP) and Cardiovascular Risk in Young Finns Study (YFS) study participants with cumulative CRP or GlycA available at the time of retinal assessment.

	STRIP	YFS
Number of participants	344	1211
Sex (% female)	54	56
Age (years, range)[a]	26 (26,26)	42 (34, 49)
Daily smoker, n (%)	22 (6.8)	152 (13)
Medication for hypertension, n (%)	5 (1.5)	115 (10)
Medication for cholesterol, n (%)	3 (0.87)	51 (4.5)
Systolic blood pressure (mmHg)	121 (11)	119 (14)
Diastolic blood pressure (mmHg)	72 (8)	75 (11)
BMI (kg/m^2)	24.3 (4.0)	26.4 (5.0)
Type 2 diabetes	0 (0)	41 (3.4)
Inflammatory bowel disease	1 (0.31)	17 (1.5)
Rheumatoid arthritis	3 (0.93)	17 (1.5)
hsCRP[b], mg/L	0.76 (0.37, 1.9)	0.74 (0.34, 1.6)
GlycA, mmol/L	1.3 (0.18)	1.0 (0.16)
Total cholesterol (mmol/l)	4.6 (0.88)	5.2 (0.96)
HDL-cholesterol (mmol/l)	1.3 (0.33)	1.3 (0.34)
LDL-cholesterol (mmol/l)	2.8 (0.74)	3.3 (0.84)
Triglycerides (mmol/l)[b]	0.90 (0.70, 1.3)	1.1 (0.75, 1.6)
Insulin (mU/l)[b]	6.9 (5.3, 9.3)	7.4 (4.4, 11.3)
Glucose (mmol/l)[b]	5.1 (4.7, 5.3)	5.3 (4.9, 5.6)

Values are means (SD) or counts (%).
All laboratory measures are analyzed from fasted serum samples.
Data on disease status was based on register data in YFS and was self-reported in STRIP.
BMI, body mass index; hsCRP, high-sensitivity *C*-reactive protein; GlycA, Glycoprotein acetyls; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, Data not available.
[a] Mean and range.
[b] Median and Q1 and Q3.

4. Discussion

This is the first study to assess associations of long-term cumulative inflammation beginning from childhood with retinal, and to our knowledge, with any microvascular bed. We found that long-term cumulative exposure to higher levels of hsCRP was associated with wider venules even after adjusting for related cardiovascular risk factors. Additionally, we found that a single 31-year prior hsCRP measure from childhood and cumulative 10-year adulthood hsCRP exposure were associated with more tortuous venules in mid-adulthood when adjusted for age and sex, and association of the CRP measure from childhood persisted after further adjustments for related cardiovascular risk factors. In contrast, cumulative exposure for hsCRP in childhood and young adulthood was associated with less tortuous venules in young adulthood. Cumulative hsCRP was not associated with arteriolar measures, and cumulative GlycA was not associated with any retinal microvascular measures.

It has been previously hypothesized that inflammation might have a role in the development of CVD and microvascular dysfunction [3,4,37]. In line, CRP is reported to be predictive of cardiovascular morbidity and mortality and is widely associated with coronary microvascular dysfunction [5,38]. Similarly, GlycA is reported to be predictive cardiovascular morbidity and mortality [19]. CRP, however, does not seem to be causally associated with CVD outcomes [38], and to our knowledge, Mendelian randomization studies on GlycA and cardiovascular outcomes are lacking. However, CRP appears to be a useful biomarker in assessing the risk for cardiovascular outcomes and reflective of upstream inflammatory activities [38].

Previously, both wider and more tortuous venules have been reported to be predictive of cardiovascular morbidity or mortality [10–12]. These retinal measures are also constantly and mainly associated with markers of inflammation [14,15,39]: previously a meta-analysis reported that age and sex adjusted cross-sectional CRP is associated with wider venules [14], and a more recent large-scale (n = 50,000) study associated increased CRP level with wider and more tortuous venules when adjusted for age, sex, smoking and socioeconomic status [15].

In the present study, cumulative hsCRP was associated with wider venular diameter in mid-adulthood (YFS) when adjusted for sex and age, and further for related cardiovascular risk factors. Similarly, cumulative hsCRP was associated with wider venules in young adulthood (STRIP) when adjusted for sex. After further adjustments the association in young adulthood slightly weakened, albeit it can be still considered as a significant association when considered the more limited number of participants in the STRIP cohort. Instead, cumulative GlycA was not associated with venular diameter in either of the cohorts. Notably, in the present study cross-sectional hsCRP was not associated with wider venules in either cohort, suggesting that assessment of cumulative exposure for hsCRP might offer additional value in detecting the association.

It is previously hypothesized that endothelial dysfunction is likely a

Table 2
Associations of cumulative high-sensitivity *C*-reactive protein (age 11–26 years) and glycoprotein acetyls (age 9–26 years) with retinal measures in young adulthood (Special Turku Coronary Risk Factor Intervention Project [STRIP]).

		Cumulative hsCRP (log)		Cumulative GlycA	
		Model 1	Model 2	Model 1	Model 2
Arteriolar diameter	Estimate (95 % CI) [n]	0.076 (−0.042, 0.19) [326]		−6.1 (−16, 4.1) [343]	
	p-value	0.21		0.24	
Arteriolar fractal dimension	Estimate (95 % CI) [n]	0.11 (−0.010, 0.23) [326]		0.019 (−0.021, 0.060) [343]	
	p-value	0.073		0.35	
Arteriolar simple tortuosity	Estimate (95 % CI) [n]	−0.085 (−0.20, 0.029) [326]		−0.013 (−0.031, 0.0050) [343]	
	p-value	0.14		0.16	
Arteriolar curvature tortuosity (log)	Estimate (95 % CI) [n]	−0.076 (−0.19, 0.038) [326]		−0.12 (−0.33, 0.10) [343]	
	p-value	0.19		0.29	
Venular diameter	Estimate (95 % CI) [n]	0.14 (0.016, 0.26) [326]	0.11 (−0.017, 0.24) [307]	9.6 (−3.6, 23) [343]	
	p-value	0.027	0.087	0.15	
Venular fractal dimension	Estimate (95 % CI) [n]	0.021 (−0.10, 0.14) [326]		0.019 (−0.025, 0.063) [343]	
	p-value	0.73		0.39	
Venular simple tortuosity	Estimate (95 % CI) [n]	−0.14 (−0.26, −0.025) [326]	−0.13 (−0.25, −0.0088) [326]	−0.011 (−0.023, 0.00096) [343]	
	p-value	0.018	0.036	0.071	
Venular curvature tortuosity (log)	Estimate (95 % CI) [n]	−0.17 (−0.28, −0.048) [326]	−0.18 (−0.30, −0.065) [326]	−0.17 (−0.37, 0.029) [343]	
	p-value	0.0058	0.0025	0.093	

HsCRP, high sensitivity *C*-reactive protein; GlycA, Glycoprotein acetyls.
The number of participants included in each analysis is indicated in square brackets.
Model 1: Adjusted for sex (and models studying associations with arteriolar fractal dimension and arteriolar and venular curvature tortuosity for STRIP intervention study group).
Model 2: Further adjusted for cumulative risk factors showing an association ($p < 0.1$) with each retinal variable from the STRIP data. Cumulative risk factor associations with retinal measures have been previously reported [25].
- venular diameter for smoking, systolic blood pressure and triglycerides.
- venular simple tortuosity for triglycerides.
-venular curvature tortuosity for insulin.

Table 3
Associations of cumulative (2001–2011) high sensitivity *C*-reactive protein and glycoprotein acetyls with retinal measures in mid-adulthood (Cardiovascular Risk in Young Finns Study [YFS]).

		Cumulative hsCRP (log)		Cumulative GlycA	
		Model 1	Model 2	Model 1	Model 2
Arteriolar diameter	Estimate (95 % CI) [n]	−0.081 (−0.14, −0.018) [1131]	−0.036 (−0.11, 0.038) [1031]	−0.11 (−0.53, 0.31) [1131]	
	p-value	0.012	0.34	0.60	
Arteriolar tortuosity (log)	Estimate (95 % CI) [n]	0.040 (−0.025, 0.10) [1131]		−0.0056 (−0.44, 0.43) [1131]	
	p-value	0.22		0.98	
Venular diameter	Estimate (95 % CI) [n]	0.074 (0.011, 0.14) [1131]	0.070 (0.0049, 0.14) [1078]	0.30 (−0.13, 0.72) [1131]	
	p-value	0.022	0.035	0.17	
Venular tortuosity	Estimate (95 % CI) [n]	0.087 (0.023, 0.15) [1131]	0.057 (−0.018, 0.13) [1080]	0.38 (−0.046, 0.81) [1131]	
	p-value	0.0076	0.14	0.080	

HsCRP, high-sensitivity *C*-reactive protein; GlycA, Glycoprotein acetyls.
The number of participants included in each analysis is indicated in square brackets.
Model 1: Adjusted for age and sex.
Model 2: Further adjusted for cumulative risk factors showing an association ($p < 0.1$) with each retinal variable from the YFS data. Associations of the cumulative risk factors with retinal measures are shown in the supplemental material.
- Arteriolar diameter for systolic blood pressure, BMI, LDL-C, triglycerides, insulin.
- Venular diameter for smoking.
- Venular tortuosity for systolic blood pressure, BMI, glucose, triglycerides.

Table 4
Associations of 31-year prior (from the year 1980) high sensitivity *C*-reactive protein with mid-adulthood retinal measures in Cardiovascular Risk in Young Finns Study (YFS).

		Model 1	Model 2
hsCRP 1980 (log)			
Arteriolar diameter	Estimate (95 % CI)	0.023 (−0.025, 0.071)	
	p-value [n]	0.35 [1173]	
Arteriolar tortuosity (log)	Estimate (95 % CI) [n]	0.0057 (−0.045, 0.056) [1173]	
	p-value	0.82	
Venular diameter	Estimate (95 % CI) [n]	0.00066 (−0.048, 0.050) [1173]	
	p-value	0.98	
Venular tortuosity	Estimate (95 % CI) [n]	0.064 (0.015, 0.11) [1173]	0.061 (0.012, 0.11) [1168]
	p-value	0.011	0.014

HsCRP, high-sensitivity *C*-reactive protein.
The number of participants included in each analysis is indicated in square brackets.
Model 1 adjusted for age and sex.
Model 2: further adjusted for cardiovascular risk factors showing an association ($p < 0.1$) with each retinal measure in 1980. Associations of the risk factors with retinal measures are shown in the supplemental material.
- venular tortuosity for systolic blood pressure.

central mechanism linking inflammation and wider retinal venular caliber, and inflammation-related venular widening may play a role in the pathogenesis of CVDs [14,39]. However, in line with Mendelian randomization studies on CRP and cardiovascular mortality, a Mendelian randomization study reported no found causal link between CRP and retinal microvascular diameters [40], suggesting that CRP might be rather marker of an upstream inflammation processes than a causal factor. Cytokines such as interleukin-6, which stimulate the production of CRP, may be among the upstream mediators driving these inflammatory pathways [41].

In the present study, results from the YFS cohort associated a single

31-year prior childhood hsCRP measure and ten-year cumulative adulthood hsCRP with more tortuous venules in mid-adulthood when adjusted for age and sex. This is in line with previously reported cross-sectional associations of CRP with adulthood retinal microvasculature [15], and the hypothesis that increased venular tortuosity might result from endothelial dysfunction and loss of microvascular autoregulation, at least partly driven by inflammation [12,15]. In the present study, however, the association of cumulative hsCRP attenuated after further adjustments for related cardiovascular risk factors. Notably, this model was adjusted for BMI, which is previously associated with both increased CRP and venular tortuosity [15,42], and therefore the dilution of the association might be due to overadjustment.

Results from the STRIP study, however, are contrary to the previous hypothesis: 15-year cumulative hsCRP in childhood and young adulthood was associated with less tortuous venules in young adulthood. Previously, cross-sectional studies in children on associations of inflammatory markers with retinal vascular tortuosity have reported a null finding [14,43], and to our knowledge, studies in young adulthood are lacking. However, CRP is not only an unambiguous inflammatory marker and participates in tissue repair and regeneration in absence of inflammation [38,41]. In STRIP, time range utilized to assess cumulative hsCRP exposure in ages 11–26 years includes adolescence, the period of developmental physical and changes [44], and it could be hypothesized that elevation of these inflammatory marker levels during adolescence might be at least partly marker for physiological upregulated tissue regeneration instead of inflammation, and therefore hsCRP did not associate with venular tortuosity in young adulthood as expected. However, in YFS single hsCRP measure from childhood (participants aged 3–18 years) was associated with more tortuous venules in mid-adulthood conflicting with this hypothesis. Additionally, associations of cumulative inflammatory markers with venular tortuosity by age group in YFS did not seem to vary, and the cumulative exposure for the youngest age group was between ages 24 and 34 years, i.e. partly at the same age as the cumulative exposure in STRIP (for hsCRP aged 11–26 years). In the present study, however, different methods were utilized to extract retinal measures between the cohorts, and methodological issues cannot be ruled out as an explanatory factor for the unexpected association. In both cohorts, however, retinal measures were extracted from zones near the optic nerve head [24,26], thus the detected difference is not probably due to different associations of hsCRP and GlycA with venular tortuosity in different retinal regions. It is also worth noting that in the present study, participants of the STRIP constructed a relatively small cohort (n = 344), and it cannot be ruled out that due to the small study size the findings contrary to the prior hypothesis might be explained simply by chance [45].

In the present study, cumulative GlycA was not associated with wider venular diameter. Previously, GlycA was reported to cross-sectionally associate with wider venules in childhood and mid-adulthood [46]. GlycA represents the integrated concentration and glycosylation of numerous acute phase proteins released in the inflammatory state [18], and GlycA likely captures different aspects of the inflammatory response than CRP [19]. Based on the results of the present study, it seems that cumulative GlycA is only weakly associated with venular microvascular measures, mainly tortuosity, in contrast to hsCRP, and it might be that inflammation upstream pathways related to hsCRP are more relevant regarding the microvasculature.

In addition to the strengths of this study we are also aware of its limitations. Fundus photos were captured only once, and therefore we were unable to assess longitudinal retinal changes. Despite long-term exposure data, the number of participants was limited especially in STRIP, and this study might be underpowered to detect some of the possible associations. Additionally, different methods were used to assess retinal measures in YFS and STRIP utilizing macula- and disc-centered fundus photos, respectively, and results of this study may be at least partly dependent on this. In the present study, GlycA was measured using the Nightingale assay, and the obtained levels may depend on the method employed [19]. Furthermore, NMR-based measurement of GlycA may be subject to confounding by elevated triglyceride levels [19,47]. The populations of the YFS and STRIP comprise White participants, and the results of this study may not be applicable to other ethnicities.

5. Conclusion

Cumulative long-term (15 and 10 years) hsCRP exposure was associated with wider venules in young and mid-adulthood, respectively, whereas associations with venular tortuosity were inconsistent. Wider retinal venules might act as a marker for cumulative inflammatory burden.

Author contributions

O.A.R., K·P., M.J. and R.T. designed the study. O.A.R. performed the statistical analyses, wrote the manuscript and contributed to the discussion. K.P., M.J., R.T., H·N., S.R., J.M., C·C., M.A., H·V., J.N., A.J., M. K., T.L., T.P.L., T.R., J.V. and O.R. reviewed and edited the manuscript and contributed to the discussion.

Data availability statement

Due to the local legal restrictions concerning the distribution of all personal information, allowance of open access to the STRIP and YFS data is not possible. Therefore, data sharing outside the study group requires a data-sharing agreement. Investigators can submit an expression of interest to the STRIP and YFS Steering Group/Data Sharing Committee (olli.raitakari@utu.fi).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2025.120595.

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