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CLINICAL RESEARCH
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Randomized 20-year infancy-onset dietary intervention, life-long cardiovascular risk factors and retinal microvasculature

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Abstract

Background and Aims Retinal microvasculature characteristics predict cardiovascular morbidity and mortality. This study investigated associations of lifelong cardiovascular risk factors and effects of dietary intervention on retinal microvasculature in young adulthood.

Methods The cohort is derived from the longitudinal Special Turku Coronary Risk Factor Intervention Project study. The Special Turku Coronary Risk Factor Intervention Project is a 20-year infancy-onset randomized controlled dietary intervention study with frequent study visits and follow-up extending to age 26 years. The dietary intervention aimed at a heart-healthy diet. Fundus photographs were taken at the 26-year follow-up, and microvascular measures [arteriolar and venular diameters, tortuosity (simple and curvature) and fractal dimensions] were derived ($n = 486$). Cumulative exposure as the area under the curve for cardiovascular risk factors and dietary components was determined for the longest available time period (e.g. from age 7 months to 26 years).

Results The dietary intervention had a favourable effect on retinal microvasculature resulting in less tortuous arterioles and venules and increased arteriolar fractal dimension in the intervention group when compared with the control group. The intervention effects were found even when controlled for the cumulative cardiovascular risk factors. Reduced lifelong cumulative intake of saturated fats, main target of the intervention, was also associated with less tortuous venules. Several lifelong cumulative risk factors were independently associated with the retinal microvascular measures, e.g. cumulative systolic blood pressure with narrower arterioles.

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Conclusions Infancy-onset 20-year dietary intervention had favourable effects on the retinal microvasculature in young adulthood. Several lifelong cumulative cardiovascular risk factors were independently associated with retinal microvascular structure.

Structured Graphical Abstract

Key Question

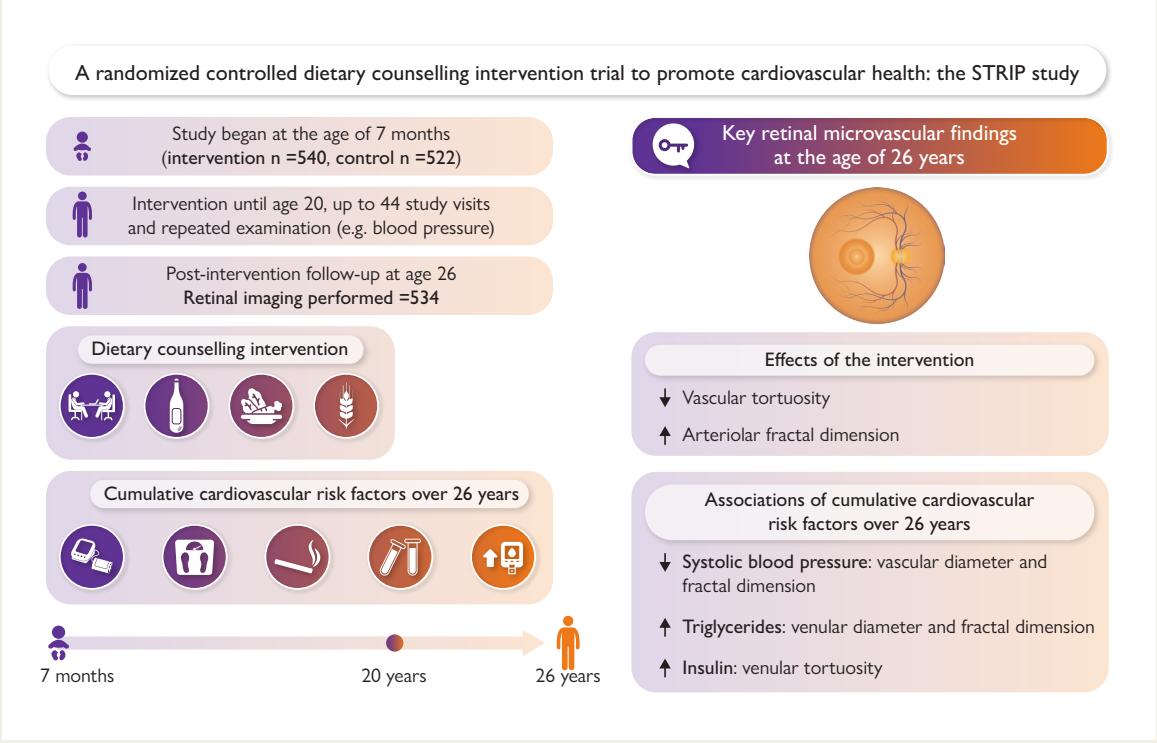
Does an infancy onset dietary counselling intervention have an effect on retinal microvasculature later in life?

Key Finding

In a cohort of 486 individuals, dietary counselling intervention had a favourable effect on retinal microvasculature. Several lifelong cardiovascular risk factors were associated with retinal microvascular alterations.

Take Home Message

Retinal microvasculature is affected already in early adulthood, and therefore microvasculature could be used to detect early vascular changes before large artery structure or function is altered.



The STRIP study. STRIP, Special Turku Coronary Risk Factor Intervention Project.

Keywords Retinal microvasculature • Microvascular • Cardiovascular health • Nutrition • Childhood • Prevention

Introduction

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality globally.[1] Although clinical manifestations of these diseases typically appear in adulthood, their roots are in early life, and childhood cardiovascular risk factors predict future cardiovascular events.[2,3]

It is hypothesized that endothelial dysfunction derived from the microvasculature may be one of the first steps of the cascade leading to CVD progression,[4,5] and early microvascular changes may provide insight into vascular health before large artery structure is altered.[6] The retinal microvasculature shares numerous characters with the systemic microcirculation[7,8] and may act as a marker of lifetime cardiovascular risk factor load,[9,10] thus offering a non-invasive approach to detect early microvascular changes. Indeed, several retinal microvascular-derived parameters are predictive for cardiovascular morbidity and mortality,[10–15] and studies in adults and children have indicated that many cardiovascular risk factors are associated with adverse microvascular structure.[5,9,16–19] Studies in children and adolescents exploring the links between long-term cardiovascular risk factors and retinal microvasculature, however, remain scarce.[20–23]

However, in the development of CVD, a pivotal role is warranted for cumulative load of the risk factors.[24–26] Despite this, cumulative associations of only blood pressure and fasting glucose on retinal vascular diameters have previously been studied, suggesting no association for glucose and an inverse association between blood pressure and retinal vascular diameters.[27,28] To our knowledge, studies on the long-term cumulative load of a wide range of conventional cardiovascular risk factors with retinal microvasculature are lacking.

Several cardiovascular risk factors are affected by diet, and suboptimal implementation of dietary recommendations is associated with increased cardiovascular morbidity and mortality.[29] To avoid these outcomes, dietary guidelines encourage e.g. intake of foods rich in unsaturated fats and avoidance of excess saturated fat. Stemming from that the atherosclerotic CVD process begins in early life and the possibility to affect cardiovascular health via diet, the Special Turku Coronary Risk Factor Intervention Project (STRIP) was launched. The STRIP is a unique 20-year dietary counselling intervention study exploring the effects of an infancy-onset dietary counselling on cardiometabolic health in early life.[30] During the 20-year intervention period, the STRIP has shown that the repeated dietary counselling aimed particularly at the replacement of saturated fat with unsaturated fat results in phenotypic changes pointing to a reduced risk of atherosclerotic CVDs and type 2 diabetes.[31–35] Despite the crucial role of the diet with cardiovascular health,[29] effects of dietary interventions on retinal microvasculature have never been reported.

The STRIP study with longitudinal data from infancy up to 26 years of age enables us to study the cumulative effects of a vast range of repeatedly measured conventional cardiovascular risk factors on multifaceted cardiovascular morbidity- and mortality-related characteristics of the retinal microvasculature.[10,11,14,15] In addition, we are able to study the effects of the unique STRIP dietary counselling intervention, reflecting dietary guidelines, on the retinal microvasculature.

Methods

Study design and participants

The randomized controlled STRIP study recruited children at 5 months of age by nurses at well-baby clinics in Turku, Finland.[30] Briefly, at the age of 7 months, 1062 infants (56.5% of the eligible age cohort; born between July 1989 and December 1991) were randomly assigned by random numbers to a dietary intervention ($n = 540$) or control ($n = 522$) group (*Figure 1*). Study group allocation was unmasked. The cohort further included two children with Down syndrome (both control), two with familial hypercholesterolaemia (intervention and control), and five children who had been randomized to the intervention group, and who missed the first study visits prior to age 13 months, and were thus later treated as controls. Additionally, a group of 45 children born between March and July 1989 was similarly recruited and randomized (intervention $n = 22$, control $n = 23$) to first test the study protocols and thus served as a 'pilot' group (later included in the final study participants).

The aim of the intervention was to reduce exposure to known environmental cardiovascular risk factors, particularly through diet.[36] Intervention families met with the counselling team, including nutritionists, nurses, and physicians at 1–3-month intervals until the child was aged 2 years, and twice per year thereafter. The control children were seen twice per year until age 7 years and annually thereafter. Similar measurements, including keeping of food diaries, were taken for both study groups, and they met the same study personnel. The intervention group received individualized dietary counselling from age 7 months until age 20 years. A fixed diet was never specified; the counselling was individualized and the child's recent food diary was used as a basis of suggestions for dietary changes. The targets of the dietary counselling intervention were based on the latest version of the Nordic nutrition recommendations. Key nutritional targets of the intervention, reflecting dietary recommendations, were a ratio of polyunsaturated and monounsaturated fat to saturated fat of more than 2:1 and an intake of saturated fat <10% of energy, cholesterol <300 mg/day (age ≥18 years), and fibre >3 g/MJ or >25 g/day (age ≥18 years). As part of the intervention, primary prevention of smoking was introduced at age eight years. A physically active lifestyle was encouraged, although it was not a structured, continuous part of the intervention.

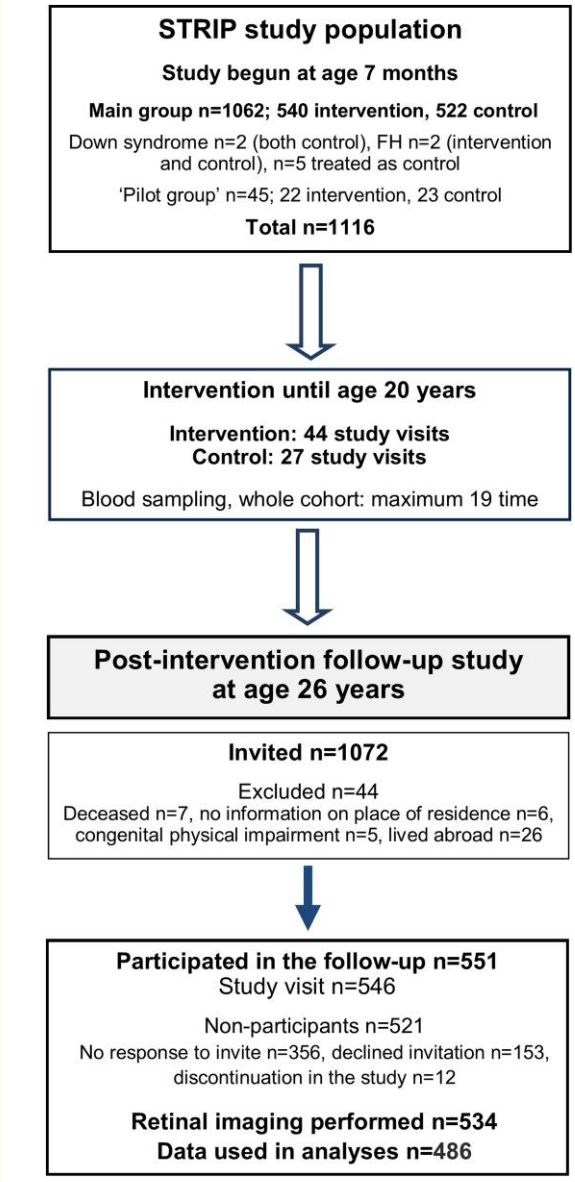


Figure 1 Flow chart of the Special Turku Coronary Risk Factor Intervention Project study from baseline to the 26-year follow-up visit. An average number of applied risk factor measurements collected during the study are given in Supplementary data online, *Table S1*. FH, familial hypercholesterolaemia; STRIP, Special Turku Coronary Risk Factor Intervention Project

The first post-intervention follow-up with the participants was conducted between April 2015 and January 2018 at the age of 26 years, 6 years after the intervention had ended (*Figure 1*).[37] Of the participating cohort ($n = 1116$), 1072 were invited to participate (excluded, $n = 44$; deceased, $n = 7$; no information on place of residence, $n = 6$; congenital physical impairment, $n = 5$; lived abroad, $n = 26$). Of them, 551 provided follow-up data (51%; intervention, $n = 263$, vs. control, $n = 288$). Of the participants, five provided only questionnaire data. Reasons for non-participation ($n = 521$) were as follows: no response to invite ($n = 356$); declined invitation ($n = 153$); and discontinuation of the study ($n = 12$). Loss to follow-up

at age 26 years has been previously reported; briefly, those who have stayed in the study have been similar to those who withdrew.[37]

Retinal measurements

At the 26-year follow-up visit, 45° digital retinal imaging was performed using a Canon non-mydriatic retinal camera (Canon CR6-45NM) fitted with a Canon 10D digital SLR camera (resolution: 3072 × 2048 pixels). Disc-centred and macula-centred images were obtained from all participants, and disc-centred images were analysed. Both eyes were photographed. Primarily right eye image of each participant was analysed. Left eye image was used if right eye image was not gradable.

Retinal images were analysed by trained graders using a semi-automated computer-based program (Singapore I Vessel Assessment, version 4, National University of Singapore, Singapore).[38,39] 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.[39] These methods are described in more details in the Supplementary data online, Supplemental material.

The analysed retina-derived variables included the following:

- Arteriolar and venular diameters using six widest arterioles and venules summarized as central retinal arteriolar equivalent (CRAE) and central retinal venular 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. This takes into account bowing and points of inflection, in contrast to simple tortuosity, which fails to distinguish between increased length due to bowing and that due to multiple points of inflection. Higher tortuosity reflects more tortuous arterioles/venules.

Cardiovascular risk factors

Standard methods were used for measuring blood pressure, serum total cholesterol, HDL cholesterol, triglycerides, as well as glucose and insulin concentrations.[37] Blood pressure, total cholesterol, and HDL cholesterol were measured at baseline and annually thereafter until age 20 years (except no blood sampling at ages 6 and 8 years; fasted samples obtained ≥5 years). Non-HDL cholesterol was calculated as total cholesterol – HDL cholesterol concentration. Triglycerides were measured since the age of 5 years, and fasting serum glucose and insulin were measured annually from age 7 to 20 years (except age 8 years).[36,37] At all annual visits, the participants' weight and height were measured and body mass index (BMI) was calculated. Since age 7 years, waist circumference was also measured at annual visits using a flexible tape measure.[34] These measurements were repeated at age 26 years.[37] Serum cotinine, a metabolic product of nicotine—used as a marker of tobacco smoke exposure—was measured annually between ages 8 and 20 years.[40,41]

Data on smoking habits were collected via questionnaires throughout the study. Regular smoking was defined as smoking at least once per day at the time of the 26-year follow-up visit (yes or no).

Diet

All participants completed a 4-day food record (3 days prior to age 2 years; consecutive, including ≥1 weekend day) before each study visit.[36] Participants were instructed to record regular days, and not e.g. holidays/sick days where food intake was atypical. At the beginning, the parents were carefully instructed to record their children's food intake. Parents and/or other caregivers were responsible for filling out the food record during infancy. As the children aged, they were given more responsibility in completing their food records; however, parents were still advised to check the records and assist the child. The food records were sent to the participants 3–4 weeks preceding the study visit with written instructions and a food portion estimation visual aid booklet to ensure accurate reporting. The control children returned their food records during the study visit, whereas the intervention children returned theirs prior to the study visit so that its nutritional composition could be calculated in advance and discussed during the study visit.[36] For both groups, the nutritionist reviewed the food records for completeness and accuracy during the visits and, if necessary, added missing details after discussion with the family. The food record data were entered into the Micro-Nutrica® food analysis software to calculate food and nutrient intakes (Research Center of the Social Insurance Institution, Turku, Finland). The software was regularly updated throughout the study period.

Cumulative cardiovascular risk factors and dietary components

Subject-specific curves for lipids (total cholesterol, HDL cholesterol, non-HDL cholesterol, and triglycerides), systolic and diastolic blood pressure, glucose and insulin, BMI, waist circumference, and dietary components [saturated fat % of energy, SAFA E%; ratio of polyunsaturated and monounsaturated fat to saturated fat, (P + M)/S ratio; and fibre, g/MJ] were estimated by mixed model regression splines.[42,43] Mixed model regression splines can handle incomplete sequences of observations and provide valid estimates under the missing at random mechanism. The age range and mean number of observations applied are shown in Supplementary data online, *Table S1*. The covariance structure for the longitudinal setting was modelled by allowing for subject-specific regression spline coefficients, which were incorporated as random effects to the model. We used three knots on the subject-specific and five knots on the fixed effects part. Because of shorter follow-up, we reduced the number of knots for triglycerides, glucose, and insulin. The mean profile was allowed to vary across sexes in terms of possibly different fixed effects parts. Similar to the approach of Lai *et al.*,[44] we then evaluated the area under the curve (AUC) as a measure of a long-term burden of the measured attributes. The approach of estimating the AUC is similar to Rovio *et al.*[42] The AUC variables were also defined separately for different age intervals of 6–8 years starting from the earliest available measurement (referred to as 'age windows').

Due to the wide intra-individual variability of cotinine concentration, we used linear interpolation to construct subject-specific curves and then evaluated the AUC. Because of missing data, participants had different follow-up durations, and therefore, the total AUC was divided by the number of follow-up years. The AUC represents thus the average cotinine levels per year throughout the age range. To control for differences in follow-up durations, participants with missing cotinine measurement at age 20 years were excluded from the analysis.

For interpretability, the AUC variables were standardized resulting in variables with mean 0 and SD 1; thus, the β coefficients indicate the amount of change in the retinal variables when the cumulative risk factor and dietary exposure increases by 1 SD. For example, 1 SD unit is equivalent to 6.2 mmHg for systolic blood pressure and 2.2 kg/m^2 for BMI when 1 SD of each AUC is divided by the duration of exposure (see Supplementary data online, *Table S1*).

Participants of this study

In total, 546 participants attended the STRIP 26-year follow-up study visit, of whom 534 participants had retinal images taken. Nine participants were excluded due to missing disc-centred image. Participants with both eyes graded as non-gradable ($n = 25$) and with venules or arterioles <6 ($n = 14$) were excluded. Overall, retinal data from 486 participants were used in the final analyses. Risk factor and dietary AUCs were available for all these participants, excluding the cotinine AUC, which was available for 295 participants. Data on regular smoking were available for 457 participants.

Statistical analysis

All continuous variables were checked for normality, and due to a right-skewed distribution arteriolar and venular curvature, tortuosity and cotinine AUC were transformed using the natural logarithmic transformation. Comparison of characteristics of those participants with retinal data vs. those who lacked the retinal data is described in the Supplementary data online, Supplemental material. Sex difference of the retinal characteristics was examined with *t*-tests.

Cumulative cardiovascular risk factors and retinal measures

Linear regression was used to assess sex-adjusted associations between standardized (mean 0, SD 1) risk factor AUCs and retinal microvasculature measures.

Study group comparisons

For these analyses, retinal variables were standardized (mean 0, SD 1). At step 1, ANOVA including sex as a confounding factor was applied. At step 2, analyses were further adjusted for related cardiovascular risk factor AUCs (*P*-value for sex-adjusted association <0.1), applying linear regression. Due to the smaller study population with cotinine AUC available, the analyses were performed primarily without cotinine AUC, but it was later included in the model. Finally, we tested the possible intervention effect modification caused by sex on retinal variables.

Cumulative dietary components

To study whether effects of the intervention were related to specific dietary components, sex-adjusted associations with standardized dietary AUCs (mean 0, SD 1) and retinal variables were studied using linear regression model. Models were further adjusted similarly as for the study group comparisons.

Multivariable models

To study confounder-adjusted (later; 'independent') cumulative risk factor exposure associations with retinal variables, each risk factor showing an association with a given retinal variable ($P < .1$), respectively, was further included in multivariable linear models. At step 1, all multivariable models were adjusted for sex and smoking, and models studying associations with retinal variables which were also affected by the intervention were conducted using study group as a factor. To avoid collinearity, only the most significant variable was included if two or more risk factor variables were closely related (e.g. systolic and diastolic blood pressure). Because of the limited data for cotinine AUC, multivariable model was conducted primarily without cotinine AUC. At step 2, cotinine AUC was included in the multivariable models to study its independent associations, and the models were no longer adjusted for smoking to avoid collinearity. Additionally, we tested the possible effect modification caused by sex on the associations between cardiovascular risk factors and those retinal variables that showed significant results in the multivariable models.

Age windows

Risk factor AUCs showing an association $P < .05$ in the multivariable models with retinal measures were divided into age windows: ages 0–6, 6–12, 12–18, and 18–26 years. Sex-adjusted age window AUC associations with related retinal measures were studied using linear regression models. Age window AUCs and retinal variables were standardized (mean 0, SD 1).

The data analyses were performed with SAS 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

Characteristics of the study population

Characteristics of the participants and their numbers in the STRIP dietary counselling intervention and control group are shown in *Table 1*. Data on the retinal characteristics of the study population are given in Supplementary data online, *Table S2*. When compared with males, females had wider arteriolar diameter and decreased arteriolar and venular fractal dimensions. Participants with applicable retinal data were similar to those who were not photographed or who were excluded from analyses, with the exception that among those who were not photographed the proportion of intervention group participants was lower compared with those with applicable retinal data (see Supplementary data online, *Table S3*).

Lifelong cumulative cardiovascular risk factors and retinal microvasculature

Increased cumulative systolic blood pressure was associated with narrower arterioles (CRAE) and venules (CRVE), and a weaker association with lower arteriolar fractal dimension was detected (*Table 2*). Diastolic blood pressure was associated with narrower arterioles but showed no other associations. In line, BMI was inversely associated with arteriolar and venular diameters. Waist circumference showed a similar, but weaker association with arteriolar diameter.

Cumulative cotinine, a metabolite product of nicotine, was associated with wider arteriolar and venular diameters, increased arteriolar fractal dimension, and weakly with decreased venular curvature tortuosity.

Cumulative HDL cholesterol was related to decreased venular fractal dimension. No associations were detected between total and non-HDL cholesterol and the retinal microvasculature measures. Triglycerides were associated with wider venules, increased venular fractal dimension, and decreased simple tortuosity. Notably, triglycerides were not associated with curvature tortuosity.

Cumulative glucose showed no associations with the retinal measures. In contrast, insulin was associated with narrower arterioles, increased venular curvature tortuosity, and weakly with decreased arteriolar fractal dimension.

Effects of the 20-year dietary intervention and associations of the cumulative dietary components

The intervention group participants had increased arteriolar fractal dimension and decreased arteriolar and venular curvature tortuosity [β .18; 95% confidence interval (CI) .02, .36, β −.24; 95% CI −.42, −.065, and β −.33; 95% CI −.51, −.16), respectively] (*Figure 2A*; adjusted for sex). These observed intervention effects remained mostly unchanged when the analyses were further adjusted for the cardiovascular risk factor AUCs also linked with the arteriolar fractal dimension and arteriolar and venular curvature tortuosity (*Figure 2B*). Additionally, after further adjusting the analyses regarding intervention effect on arteriolar fractal dimension and venular curvature tortuosity for cotinine AUC, the results remained essentially unchanged (β .19; 95% CI −.049, .42, and β −.36; 95% CI −.59, −.13, respectively). There was no significant ($P < .05$) effect modification caused by sex (data not shown), and therefore, effects of the intervention were not further studied by sex.

Associations of dietary AUCs, indicative of the STRIP intervention targets, with the retinal measures are shown in *Table 3*. The cumulative intake of SAFA (E%) was directly associated with venular curvature tortuosity. In line, a higher intake of (*P* + M)/S, indicative of a more favourable composition of dietary fats, was associated with less tortuous venules. When further adjusted for venular curvature tortuosity-related risk factor AUCs (insulin and cotinine), the results remained essentially

Table 1 Characteristics of participants with applicable retinal data at the 26-year follow-up

	Intervention	Control	All
Number of participants with gradable fundus photograph	237	249	486
Age (years)	26.1 (0.2)	26.1 (0.2)	26.1 (0.2)
Sex (% female)	51.5	59.4	55.6
Daily smoker (%)	23 (10.3)	21 (9.0)	44 (9.6)
Familial hypercholesterolaemia (%)	1 (0.4)	2 (0.8)	3 (0.6)
Type 1 diabetes (%)	3 (1.3)	2 (0.8)	5 (1.0)
Type 2 diabetes (%)	0 (0)	0 (0)	0 (0)
Antihypertensive medication (%)[a]	4 (1.7)	3 (1.2)	7 (1.4)
Lipid-lowering medication (%)[a]	3 (1.3)	2 (0.8)	5 (1)
Systolic blood pressure (mmHg)	121.9 (10.8)	120.2 (11.0)	121.0 (10.9)
Diastolic blood pressure (mmHg)	72.0 (7.6)	72.0 (7.3)	72.0 (7.5)
BMI (kg/m^2)	24.5 (4.0)	24.4 (4.7)	24.4 (4.3)
Waist (cm)	81.2 (10.8)	80.8 (11.7)	81.0 (11.3)
Total cholesterol (mmol/L)	4.5 (0.9)	4.7 (0.9)	4.6 (0.9)
HDL cholesterol (mmol/L)	1.3 (0.3)	1.4 (0.3)	1.3 (0.3)
LDL cholesterol (mmol/L)	2.7 (0.7)	2.9 (0.8)	2.8 (0.8)
Triglycerides (mmol/L)[b]	0.9 (0.7, 1.3)	0.9 (0.7, 1.2)	0.9 (0.7, 1.3)
Insulin (mU/L)[b]	6.6 (5.2, 9.1)	7.2 (5.4, 9.4)	6.9 (5.3, 9.2)
Glucose (mmol/L)	5.1 (1.1)	5.1 (0.5)	5.1 (0.8)

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Values are means (SD) or counts (%).
All laboratory measures are analysed from fasted serum samples.
The population of the STRIP study comprises White participants.
[a]Data obtained from a self-reported questionnaire.
[b]Median and lower and upper quartiles.

unchanged (β .024; 95% CI .00011, .047, and β −.020; 95% CI −.044, .0033, respectively).

Multivariable analyses

Multivariable associations of those cumulative risk factors associated with the retinal measures ($P < .1$; *Table 2*) were analysed to study independent associations of the cumulative risk factors and each retinal variable (*Table 4*). Increased cumulative systolic blood pressure was independently associated with the arteriolar diameter, whereas association of insulin with arteriolar diameter weakened and association of BMI was lost. Systolic blood pressure was also inversely associated with the venular diameter and arteriolar and venular fractal dimensions. Triglycerides were associated with increased venular diameter and fractal dimension, while HDL cholesterol was no longer associated with the venular fractal dimension. Insulin was independently associated with increased venular curvature tortuosity.

Due to the smaller amount of data available, the multivariable associations of cumulative cotinine with the retinal measures were investigated separately (see Supplementary data online, *Table S4*). Cotinine was associated with wider arteriolar and venular diameters and increased arteriolar fractal dimension, while the association with decreased venular curvature tortuosity diluted.

Additionally, effect modification caused by sex was tested for the cumulative risk factors independently associated with the retinal measures. The analyses revealed no significant ($P < .05$) effect modification caused by sex (data not shown), and therefore, the risk factor associations were not further studied by sex.

Age windows

When analysed by age windows, greater systolic blood pressure exposure in early childhood, i.e. between ages 0 and 6 years, was already associated with narrower arteriolar diameter and the association became even stronger as the participants aged (*Figure 3*). Systolic blood pressure in early childhood was not associated with venular diameter and arteriolar and venular fractal dimensions, but the associations became evident with advancing age.

Insulin was associated with more tortuous venules in all age windows. Triglycerides, beginning from the age of 5 years, were associated in all age windows with increased venular diameter and fractal dimension.

Discussion

This is the first study to assess the effect of dietary intervention on retinal microvasculature showing that the infancy-onset intervention had

Table 2 Associations of cumulative cardiovascular risk factor exposure with retinal measures (analyses adjusted for sex)

		Arteriolar				Venular			
		CRAE	Fractal dimension	Simple tortuosity	Log curvature tortuosity	CRVE	Fractal dimension	Simple tortuosity	log Curvature tortuosity
Blood pressure									
Systolic	β estimate	−2.82	−0.0044	−.00014	.012	−1.84	−.0043	−.00042	.011
	95% CI	−3.92, −1.71	−.0089, .000012	−.0022, .0019	−.013, .037	−3.25, −.43	−.0092, .00046	−.0018, .00096	−.011, .033
	P-value	<.0001	.056	.90	.33	.011	.077	.55	.34
Diastolic	β estimate	−2.22	−.0028	−.00092	.0060	−1.05	−.0029	−.00041	.0040
	95% CI	−3.24, −1.20	−.0070, .0013	−.0028, .00098	−.017, .029	−2.35, .25	−.0074, .0015	−.0017, .00086	−.017, .024
	P-value	<.0001	.18	.34	.61	.11	.19	.53	.70
Exposure to tobacco smoke									
log Cotinine	β estimate	1.42	.0075	.0020	.0043	2.11	.0017	−.00054	−.024
	95% CI	.030, 2.80	.0020, .013	−.00040, .0044	−.025, .033	.35, 3.87	−.0042, .0076	−.0022, .0011	−.050, .0023
	P-value	.045	.0077	.10	.77	.019	.57	.53	.074
Anthropometrics									
BMI	β estimate	−1.36	−.00015	−.00090	−.0035	−1.21	.00050	.000036	.0071
	95% CI	−2.40, −.32	−.0043, .0040	−.0028, .0010	−.027, .020	−2.51, .091	−.0039, .0049	−.0012, .0013	−.013, .028
	P-value	.010	.94	.36	.76	.068	.83	.96	.50
Waist	β estimate	−1.043	−.0011	.00033	.0034	−.59	.0020	.00017	.097
	95% CI	−2.15, .068	−.0056, .0034	−.0017, .0024	−.021, .028	−1.98, .81	−.0027, .0068	−.0012, .0015	−.012, .032
	P-value	.066	.63	.75	.79	.41	.40	.80	.39

Continued

Table 2 *Continued*

		Arteriolar				Venular			
		CRAE	**Fractal dimension**	**Simple tortuosity**	**Log curvature tortuosity**	**CRVE**	**Fractal dimension**	**Simple tortuosity**	**log Curvature tortuosity**
Lipids									
Total-C	β estimate	−.028	−.00031	−.0000034	.015	−.57	−.0025	−.00026	.0042
	95% CI	−1.095, 1.038	−.0046, .0040	−.0020, .0019	−.0083, .039	−1.90, .77	−.0070, .0020	−.0016, .0010	−.017, .025
	P-value	.96	.89	.97	.20	.41	.28	.69	.70
non-HDL-C	β estimate	.056	−.00046	.00019	.016	−.29	−.0013	−.00019	.0057
	95% CI	−1.00, 1.11	−.0047, .0037	−.0017, .0021	−.0068, .040	−1.61, 1.024	−.0058, .0031	−.0015, .0011	−.015, .026
	P-value	.92	.83	.84	.17	.66	.56	.77	.59
HDL-C	β estimate	−.29	.00046	−.00056	−.0016	−.96	−.0044	−.00018	−.0035
	95% CI	−1.37, .79	−.0039, .0048	−.0025, .0014	−.026, .022	−2.31, .39	−.0090, .00016	−.0015, .0011	−.025, .018
	P-value	.59	.83	.58	.90	.16	.059	.78	.75
Triglycerides	β estimate	.29	.0027	.00022	.0032	1.41	.0061	−.0014	−.0063
	95% CI	−.75, 1.34	−.0015, .0069	−.0017, .0021	−.020, .026	.11, 2.71	.0017, .010	−.0027, −.00018	−.027, .014
	P-value	.58	.20	.82	.79	.034	.0071	.025	.55
Glucose metabolism									
Glucose	β estimate	−.38	−.0023	−.0017	−.017	.45	−.0020	−.00067	−.0046
	95% CI	−1.53, .76	−.0068, .0023	−.0038, .00038	−.042, .0086	−.98, 1.89	−.0069, .0029	−.0021, .00072	−.027, .018
	P-value	.51	.34	.11	.19	.53	.41	.34	.69
Insulin	β estimate	−1.49	−.0035	−.00054	.0063	.35	.0014	−.000013	.023
	95% CI	−2.53, −.45	−.0077, .00070	−.0025, .0014	−.017, .030	−.97, 1.66	−.0031, .0058	−.0013, .0013	.0024, .043
	P-value	.0050	.10	.58	.60	.61	.55	.98	.029

BMI, body mass index; CI, confidence intervals; CRAE, central retinal arteriole equivalent; CRVE, central retinal venular equivalent; HDL-C, high-density lipoprotein cholesterol; log, logarithmic; non-HDL-C, non-high-density lipoprotein cholesterol; Total-C, total cholesterol.
All laboratory measures are analysed from serum samples.
The cumulative cardiovascular risk factor exposure was calculated as a risk factor-specific area under the curve (AUC).
The sex-adjusted β estimates, 95% Cis, and *P*-values are from linear models and refer to change in the retinal variables associated with 1-SD change in the standardized (mean 0, SD 1) risk factor AUC.

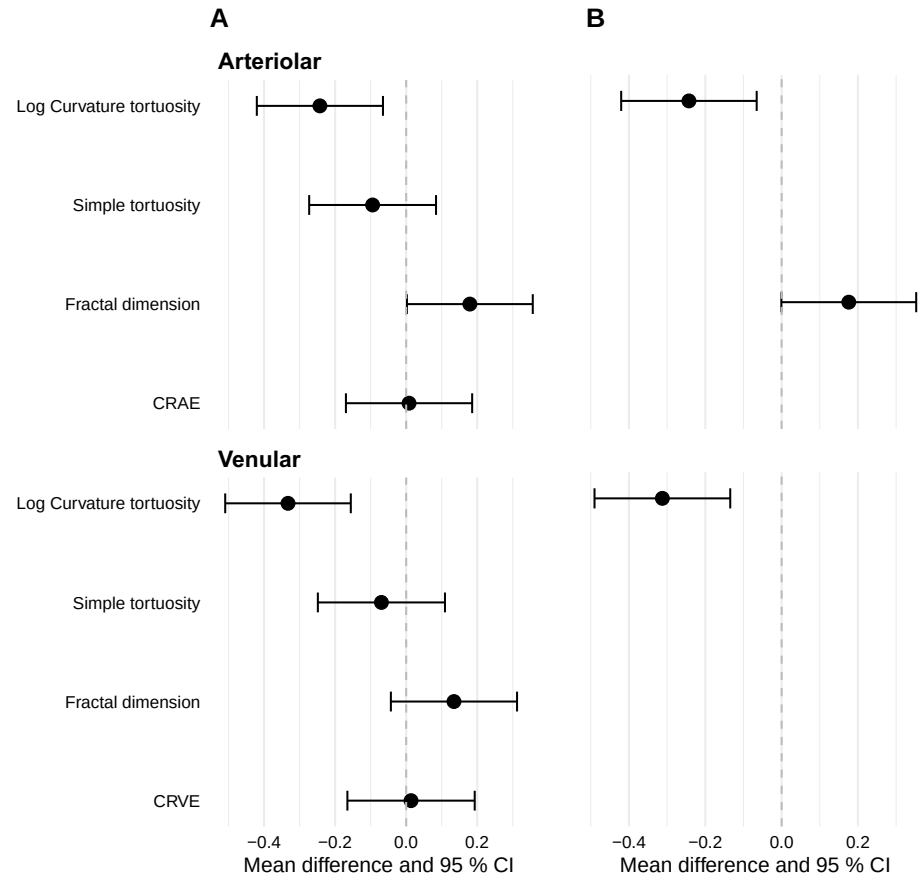


Figure 2 Effects of the intervention on retinal measures. (*A*) Sex-adjusted effect of the intervention. (*B*) Retinal variables showing intervention effects in (*A*) further adjusted for related cumulative cardiovascular risk factors from *Table 2*. The model studying the effects of the intervention on arteriolar fractal dimension was further adjusted for systolic blood pressure and venular curvature tortuosity for insulin, while no further adjustment was done for arteriolar curvature tortuosity due to no related cumulative risk factors. The cumulative cardiovascular risk factor exposure was calculated as a risk factor-specific area under the curve. Due to limited data for cotinine area under the curve, (*B*) is not adjusted for cotinine. The circle indicates the mean difference in standardized retinal measures (mean 0, SD 1) between the intervention and control groups (control group as the reference). CI, confidence interval; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent

favourable effects on retinal microvascular structure. In addition, data on the associations of lifelong exposure to cumulative cardiovascular risk factors on microvasculature have not been reported. To our knowledge, this is also the first study to report broadly the association of lifelong cumulative risk factors starting from infancy with any vascular phenotype. Previously, both cumulative cardiovascular risk factors and retinal microvasculature have been reported to be predictive for cardiovascular events,[10–15,24–26] and cumulative childhood risk factors have been associated with left ventricular remodelling and carotid plaque in adulthood.[44,45] In this study, lifelong exposure to several cumulative cardiovascular risk factors was associated with morbidity- and mortality-related retinal measures in early adulthood, and some of these associations were evident since early childhood (see *Structured Graphical Abstract*).

In this study, both cumulative systolic and diastolic blood pressures were strongly associated with decreased arteriolar diameter, and systolic blood pressure was also inversely associated with venular diameter and both the arteriolar and venular fractal dimensions. Moreover, the association with arteriolar diameter was evident since early childhood. All the associations between systolic blood pressure and retinal measures remained when further adjusted in multivariable analyses, indicating that systolic blood pressure likely plays a major role in microvasculature structure. Previously, an inverse association between blood pressure and arterioles has been widely reported.[17] More recently, a large-scale study ($n = 55\,000$) reported that both systolic and diastolic blood pressures are associated with decreased arteriolar and venular diameters and increased tortuosity.[9] In addition, decreased fractal dimension is associated with prevalent and incident hypertension.[14] Moreover, an increased cumulative systolic blood pressure was reported to be associated with narrower arterioles,[27] whereas another study reported an inverse association between cumulative diastolic, but not systolic, blood pressure and arteriolar as well as venular diameters.[28] Our results were in line with the observed associations in these studies, supporting that cumulative blood pressure is associated with decreased microvascular diameters. In addition, childhood systolic blood pressure is previously associated with decreased arteriolar

Table 3 Associations of cumulative dietary components and retinal measures (analyses adjusted for sex)

		Arteriolar measures				Venular measures			
		CRAE	Fractal dimension	Simple tortuosity	Log Curvature tortuosity	CRVE	Fractal dimension	Simple tortuosity	Log Curvature tortuosity
SAFA	β estimate	.26	−.0013	.00044	.016	1.09	−.0028	.00058	.029
	95% CI	−.78, 1.30	−.0054, .0029	−.0015, .0023	−.0074, .039	−.21, 2.39	−.0072, .0016	−.00068, .0018	.0083, .049
	P-value	.63	.55	.65	.18	.10	.21	.36	.0057
(P + M)/S	β estimate	−.26	.0031	−.00058	−.0094	−.44	.0015	−.00046	−.021
	95% CI	−1.31, .79	−.0011, .0073	−.0025, .0013	−.033, .014	−1.75, .87	−.0029, .0060	−.0017, .00082	−.042, −.00056
	P-value	.63	.14	.56	.42	.51	.50	.48	.044
Fibre	β estimate	.36	.00093	−.0015	−.015	−.41	−.00077	−.00031	−.016
	95% CI	−.76, 1.47	−.0035, .0054	−.0036, .00051	−.040, .096	−1.80, .99	−.0055, .0040	−.0017, .0010	−.038, .0057
	P-value	.53	.68	.14	.23	.57	.75	.65	.15

CI, confidence interval; CRAE, central retinal arteriole equivalent; CRVE, central retinal venular equivalent; SAFA, intake of saturated fat as % of energy; (P + M)/S, ratio of polyunsaturated and monounsaturated fat to saturated fat. Fibre, intake as g/MJ.
The cumulative dietary component was calculated as a risk factor-specific area under the curve (AUC).
The sex-adjusted β estimates, 95% CIs, and *P*-values are from linear models and refer to change in the retinal variables associated with 1-SD change in the standardized (mean 0, SD 1) dietary AUC.

diameter and increased tortuosity in mid-adulthood.[22] When considered that the follow-up in this study reaches early adulthood, it might be that arteriolar tortuosity increases slowly and becomes evident later in life.

Cumulative cotinine was independently associated with wider arterioles and venules and increased arteriolar fractal dimension. These findings are in line with previous literature studying associations between smoking status and retinal microvasculature.[46,47] Arteriolar widening is hypothesized to derive from smoking-related hypoxia, and angiogenic effects of nicotine might explain increased fractal dimension.[46,47] Venular widening might be caused by smoking-related inflammation.[46]

In this study, cumulative sex-adjusted BMI and waist circumference were inversely associated with arteriolar diameter, and BMI was also associated with decreased venular diameter. When further adjusted in multivariable analyses, these associations diluted. However, other risk factors included in the same model, such as systolic blood pressure, might be mediating the association. In an adult population, narrower arterioles as well as wider and more tortuous venules have been associated with greater BMI and waist circumference, and a wider venular diameter was most strongly associated with total fat percentage.[48] However, a meta-analysis conducted on children and adolescents reported that childhood obesity is predominantly associated with retinal arteriolar narrowing, and the association of BMI with wider venules might be inconsistent.[19] In line with our results, decade-long BMI and waist circumference trajectories in children were associated with narrower arterioles, but not with venular widening.[23] Wider venules have been associated with inflammation.[18] Previously in children, higher BMI was associated with narrower arterioles, whereas C-reactive protein was associated with wider venules,[49] and it can be hypothesized that obesity-related inflammation becomes evident in retinal venules later in life.

In the present study, cumulative triglycerides were directly associated with venular diameter and fractal dimension and inversely with venular simple tortuosity. Notably, curvature tortuosity showed no association. In addition, cumulative HDL cholesterol was associated with decreased venular fractal dimension, although this association was lost in the multivariable analyses. No other associations between cumulative lipids and retinal microvasculature were detected. A large-scale study has previously associated cholesterol and triglycerides levels with decreased arteriolar diameter, LDL cholesterol, and triglycerides with increased venular diameter and triglycerides with more tortuous arterioles in a non-diabetic population.[18] In contrast, in children and adolescents results on the vessel diameters are somewhat mixed, and it is hypothesized that the detected associations developed gradually later in adolescence.[5] This is in line with our results, showing retinal associations only with the cumulative triglycerides.

In this study, cumulative glucose was not associated with any retinal measure, but the sex-adjusted insulin was associated with decreased arteriolar diameter and fractal dimension and more tortuous venules. Furthermore, the association with venular tortuosity was evident since the earliest available age window. In the multivariable analysis, insulin was independently associated with venular tortuosity, whereas the association with arteriolar diameter was less pronounced. When considered that hyperinsulinaemia is reported to predict hypertension, weakened association between insulin and arteriolar diameter might at least partly be due to mediation.[50] Previously, cumulative glucose was not associated with retinal vessel diameters,[27] and to our knowledge, associations of cumulative insulin with microvasculature have not been previously studied. Recently, a large-scale cross-sectional study reported that both increased HbA1c and type 2 diabetes are

Table 4 Associations of cumulative cardiovascular risk factor exposures and retinal measures estimated by multivariable regression analysis

		Arteriolar		Venular		
		CRAE	Fractal dimension	CRVE	Fractal dimension	Log curvature tortuosity
Systolic blood pressure	β estimate (95% CI)	−2.69 (−3.92, −1.46)	−.0051 (−.0097, −.00048)	−1.78 (−3.30, −.26)	−.0055 (−.010, −.00055)	#
	P-value	<.0001	.031	.022	.029	#
BMI	β estimate (95% CI)	−.14 (−1.32, 1.04)	#	−1.00 (−2.44, .44)	#	#
	P-value	.81	#	.17	#	#
HDL cholesterol	β estimate (95% CI)	#	#	#	−.0023 (−.0071, .0025)	#
	P-value	#	#	#	.34	#
Triglycerides	β estimate (95% CI)	#	#	1.51 (.15, 2.87)	.0055 (.00077, .010)	#
	P-value	#	#	.030	.023	#
Insulin	β estimate (95% CI)	−.99 (−2.16, .19)	#	#	#	.023 (.0011, .046)
	P-value	.099	#	#	#	.040

AUC, area under the curve; BMI, body mass index; CI, confidence interval; CRAE, central retinal arteriole equivalent; CRVE, central retinal venular equivalent; HDL, high-density lipoprotein.
All laboratory measures are analysed from fasted serum samples.
The cumulative cardiovascular risk factor exposure was calculated as a risk factor-specific area under the curve (AUC).
This table shows multivariable associations of risk factor AUCs with retinal variables. Only risk factor AUC, which showed sex-adjusted associations (*Table 2*, *P*-value < .1) with each retinal variable, was included in the analyses. For example, systolic blood pressure, BMI, and insulin were associated with CRAE and therefore included in the model (*Table 2*, *P*-values < .0001, .010, and .0050, respectively). Due to limited data for cotinine AUC, analyses were primarily conducted without cotinine AUC, which was later added to the analyses (see Supplementary data online, *Table S4*). All models were adjusted for sex and smoking status. Additionally, retinal variables showing intervention effect (arteriolar fractal dimension and arteriolar and venular curvature tortuosity) were adjusted for the study group. For example, in *Table 2* only systolic blood pressure was associated with arteriolar fractal dimension (*P*-value .056), and therefore, the multivariable table is showing association of systolic blood pressure with arteriolar fractal dimension after confounder adjustment by sex, smoking status, and study group.
The β estimates, 95% CIs, and *P*-values are from multivariable linear models and refer to change in the retinal variables associated with 1-SD change in the standardized (mean 0, SD 1) risk factor AUC.
#, not included in the analysis.

associated with more tortuous venules.[18] Stemming from the results of the present study, it might be that more tortuous venules are associated with diabetic pathogenic processes including insulin resistance and hyperinsulinaemia, rather than being affected mainly due to increased glucose levels.

Uniquely, we were able to study the effects of the 20-year infancy-onset dietary counselling intervention on early adulthood microvasculature. We found that participants in the intervention group had less tortuous arterioles and venules and increased arteriolar fractal dimension when compared with the control group. These associations remained unchanged when further adjusted for cumulative risk factors. According to previous studies, these retinal measures are related to cardiovascular morbidity and mortality,[14,15,18] suggesting that the intervention had independent of these risk factors, a protective effect on cardiovascular health. This effect might be due to better microvascular endothelial function.[4]

Previously, deep learning of the retinal fundus images for the prediction of major adverse cardiovascular events achieved similar accuracy to the composite European SCORE risk calculator, and the neural network model paid attention to the vascular regions in the retina, highlighting the importance of retinal vessel structure in predicting for cardiovascular events.[51] Stemming from these findings, and what we have previously reported, it is likely that participants in the STRIP intervention group have reduced risk of cardiometabolic diseases.[37] It might be assumed that retinal microvasculature offers a window into cardiovascular risk assessment, which is unreachable even for the lifelong exposure for multiple conventional CVD risk factors.

Earlier studies on diet quality and retinal microvasculature are scarce, and their quality has been regarded as low to medium.[52] To better understand effects of the intervention, lifelong dietary component AUCs, reflecting the key intervention targets, were studied. Cumulative intake of saturated fatty acids was associated with more tortuous venules, even when further adjusted for cumulative risk factors. Similarly, an association of more favourable quality of fat with less tortuous venules was also detected. When noted that in the intervention group the intake of saturated fat is lower, it can be concluded that the effect of the intervention on venular tortuosity may partly be mediated by more favourable quality of dietary fats—the main target of the intervention.[37] Interestingly, our data showed no associations with cumulative lipids and venular tortuosity, suggesting a different pathway between dietary fat composition and venular tortuosity.

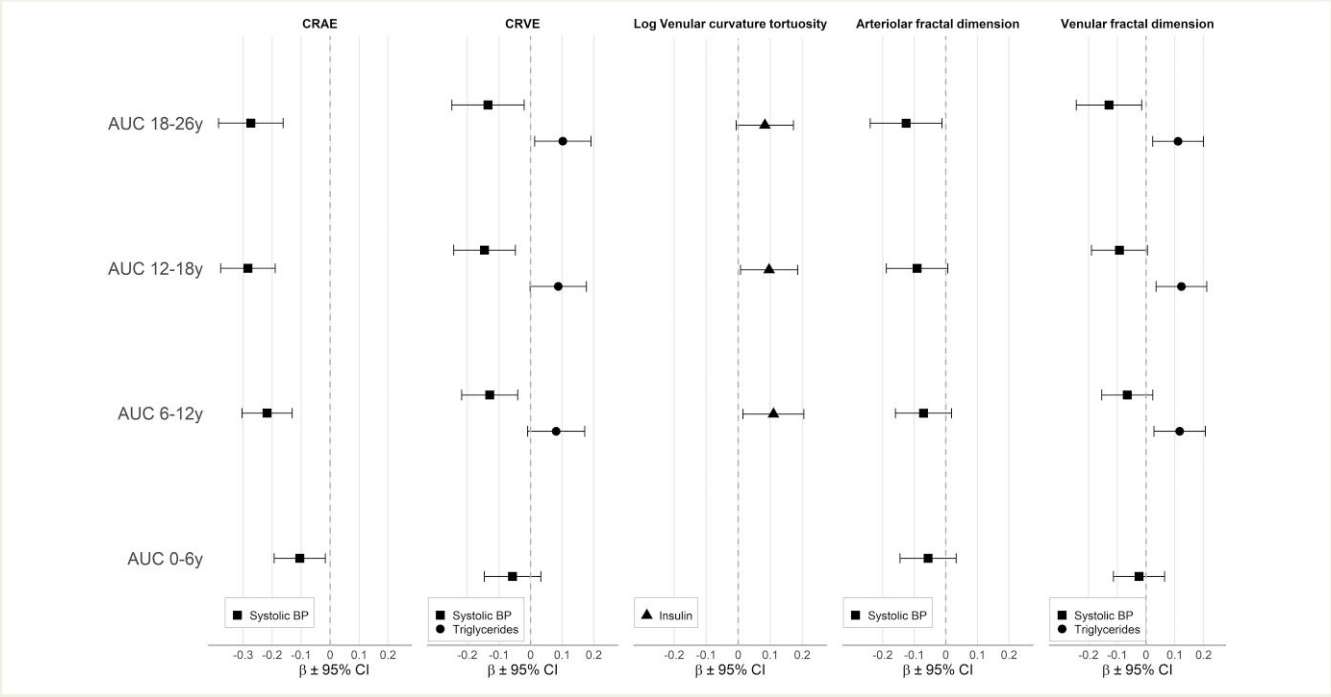


Figure 3 Associations of cumulative cardiovascular risk factors with retinal measures in different age windows. The cumulative cardiovascular risk factor exposure was calculated as a risk factor-specific area under the curve. Risk factor areas under the curves showing an association $P < .05$ in the multivariable models (*Table 4*) and related retinal variables are included in this figure. Risk factor areas under the curves were divided into 6–8-year intervals starting from the earliest available measurement to study associations of cumulative risk factor exposure from early life to young adulthood with retinal variables. All models are adjusted for sex. All laboratory measures are analysed from serum samples. AUC, area under the curve; BP, blood pressure; CI, confidence interval; CRAE, central retinal arteriole equivalent; CRVE, central retinal venular equivalent

In contrast to our previous studies,[33,53,54] retinal microvasculature was the only vascular phenotype distinguishing the intervention and control groups thus far. This finding is in line with the hypothesis that microvasculature might be the first responder for adverse effects of endothelial dysfunction, and large artery changes may arise later in life.[4–6] Future follow-ups of the STRIP study will provide data on whether the intervention confers long-term cardiometabolic disease risk reduction.

Limitations

In addition to several strengths of the present study, we are also aware of its limitations. Despite the repeatedly measured risk factors used to assess cumulative associations, the cohort size of this study was relatively small, and it might be unpowered to detect some of the associations. Despite the longitudinal risk factor and dietary data, fundus photographs were available only at the 26-year follow-up, and we were unable to assess longitudinal changes in the microvasculature. The number of intervention group participants was lower among those for whom the retinal photograph was not taken. However, only 12 participants lacked the photographs, while applicable retinal data were obtained from 89% study visit participants. Due to the young age of study participants and the continuous nature of retinal measures with no available cut-points, we were unable to study intervention effects on clinically meaningful endpoints considered as events. Despite the standardized protocols, the retinal vasculature grading may include errors related to the grader, image quality variability, and unknown issues (e.g. pulse cycle), which may lead to less precise measurements. Retinal microvascular measures were not defined as outcomes in the original study protocol and were later introduced to the study. Several grading tools have been used to extract retinal vascular data from fundus photographs,[10,13–15] and it might be that results of these studies are at least partly modified by different grading tools used to analyse retinal microvasculature and its associations with cardiovascular morbidity and mortality.[55] Therefore, predictive values of retinal measures reported in previous studies may not be fully applicable to assess effects of the intervention on cardiovascular health. The population of the STRIP study comprises White participants. While these detected associations are probably robust among White individuals, they may not be applicable to other ethnic groups.

Conclusions

The 20-year infancy-onset dietary intervention has an independent and favourable effect on early adulthood retinal microvasculature. Our study also suggests that several lifelong cumulative cardiovascular risk factors are associated with early adulthood microvasculature, and some of these associations are evident since early childhood.

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The STRIP study children and their parents and grandparents have made the study possible—the authors thank them for their time, continued efforts, and commitment to the STRIP throughout the years.

Supplementary data

Supplementary data are available at *European Heart Journal* online.

Declarations

Disclosure of Interest

M.J. received payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing, or educational events from Amgen and Sanofi and participation on a Data Safety Monitoring Board or Advisory Board of Sanofi. The remaining authors have nothing to declare.

Data Availability

Selected variables and their descriptions without personal identification codes are distributed to investigators and collaborators working on specific projects. The rights to the data belong to the STRIP research group. Data sharing outside the STRIP group requires a data sharing agreement. Investigators can submit an expression of interest to the STRIP Steering Committee.

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Ethical Approval

The study was approved by the associated university and hospital district ethical authorities. Written informed consent was obtained from parents at study entry and from the participants at ages 15, 18, and 26 years.

Pre-registered Clinical Trial Number

The STRIP study is registered at ClinicalTrials.gov, NCT00223600, https://www.clinicaltrials.gov.

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