

Retinal microvascular function and incidence and trajectories of clinically relevant depressive symptoms: The Maastricht Study

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Methods S1. Definition of major depression

Clinically relevant depressive symptoms, as assessed by the 9-item Patient Health Questionnaire (PHQ-9), can occur both in the presence or in the absence of a clinical diagnosis of major depression according to the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DMS-4) criteria for a major depressive episode. Individuals without clinically relevant depressive symptoms can also have a major depression diagnosis according to the DMS-4 criteria for a major depressive episode. At the baseline examination only, presence of a clinical diagnosis of major depression according to the DSM-4 criteria for a major depressive episode was assessed using the Mini-International Neuropsychiatric Interview (MINI).(Sheehan et al., 1998) The MINI is a short diagnostic structured interview used to assess presence of a current or lifetime diagnosis of major depression. A diagnosis of major depression is defined as 1) one core symptom (i.e. depressed mood or loss of interest) and at least four other symptoms of depression (i.e. significant weight change or change in appetite, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, guilt or worthlessness, diminished ability to think or concentrate or indecisiveness and suicidal thoughts or plans), or 2) two core symptoms of depression and at least three other symptoms, for a period of more than two weeks.

Methods S2. Definition of covariates

We assessed age (years), sex (male/female), socioeconomic status, smoking status, alcohol use, dietary habits and prior cardiovascular disease (yes/no) by questionnaire. Socioeconomic status was assessed using education and income. Education was classified into three groups: low (none, primary or lower vocational education only), intermediate (intermediate general secondary, intermediate vocational or higher general secondary education) and high (higher vocational education or university level of education). Income was calculated as the household income divided by the square root of household size. Smoking status was categorized in never, former and current smoker. Alcohol use was defined as non-consumer, low consumer (≤ 7 alcoholic drinks/week for women; ≤ 14 alcoholic drinks/week for men) or high consumer (> 7 alcoholic drinks/week for women; > 14 alcohol drinks/week for men). Dietary habits were assessed with the Dutch Healthy Diet index sum score, a measure of adherence to the Dutch dietary guidelines 2015.(Looman et al., 2017) The Dutch Healthy Diet sum score was calculated as described previously (count with a range from 0 to 150).(Looman et al., 2017)

In the present study, we excluded alcohol use as one of the components of the score, because we adjusted for alcohol use using a separate variable, as done previously.(van der Heide et al., 2021) Medication use (yes/no) was assessed from medication boxes brought to the clinic.(Schram et al., 2014) Moderate-to-vigorous physical activity (hours/week) was assessed by the Champs physical activity questionnaire (Harada, Chiu, King, & Stewart, 2001) and by accelerometry (activPAL3 physical activity monitor (Vandercappellen et al., 2022)). Glucose metabolism status, blood pressure (mmHg; office and 24-h), body mass index (kg/m²), waist circumference (cm), total-to-HDL cholesterol ratio and markers of low-grade inflammation were measured using standardized methods.(Schram et al., 2014) We defined hypertension as an office blood pressure of $\geq 140/90$ mmHg, antihypertensive medication use, or both. We used a two-hour oral glucose tolerance test (Schram et al., 2014) to classify participants as having normal glucose metabolism, prediabetes (i.e. impaired fasting glucose and/or impaired glucose tolerance) or type 2 diabetes based on the World Health Organization 2006 criteria.(World Health Organization, 2006) Plasma markers of low-grade inflammation were measured as described previously,(Vandercappellen et al., 2022) and included C-reactive protein, serum amyloid A, tumor necrosis factor- α , interleukin-6 and interleukin-8. The plasma markers were summarized into a composite score of low-grade inflammation, as done previously.(Vandercappellen et al., 2022)

Methods S3. Example code of traj-command in Stata

For the trajectory analysis we used the traj-command in Stata with the following code:

```
traj, var(varlist) indep(varlist) model(string) order (numlist)
```

var(*varlist*) dependent variables, measured at different times (i.e. clinically relevant depressive symptoms)

indep(*varlist*) independent variables, i.e. when the dependent variables were measures (i.e. annual waves of data collection)

model(*string*) probability distribution for the dependent variables (i.e. logit)

order(*numlist*) polynomial type (0=intercept, 1=linear, 2=quadratic, 3=cubic) for each group.

Table S1. Characteristics of the total study population on flicker light-induced retinal dilation, and according to incident clinically relevant depressive symptoms and trajectories clinically relevant depressive symptoms

	Total study population (n=4,744)	According to clinically relevant depressive symptoms*		Trajectories of change of clinically relevant depressive symptoms†			
		No incident depressive symptoms (n=4,122, 86.9%)	Incident depressive symptoms (n=622, 13.1%)	Low (n=4,185, 88.2%)	Early-chronic (n=121, 2.6%)	Late-increasing (n=189, 4.0%)	Remitting (n=249, 5.3%)
Demography							
Age, years	59.6 (8.5)	59.6 (8.5)	59.4 (9.0)	59.6 (8.5)	59.3 (8.1)	60.4 (9.5)	58.9 (9.0)
Sex							
Female, No (%)	2,393 (50.4)	2,059 (50.0)	334 (53.7)	2,094 (50.0)	63 (52.1)	101 (53.4)	135 (54.2)
Male, No (%)	2,351 (49.6)	2,063 (50.0)	288 (46.3)	2,091 (50.0)	58 (47.9)	88 (46.6)	114 (45.8)
Education‡							
Low, No (%)	1,496 (31.9)	1,243 (30.5)	253 (41.5)	1,263 (30.5)	54 (45.8)	72 (38.9)	107 (43.7)
Intermediate, No (%)	1,337 (28.5)	1,167 (28.6)	170 (27.9)	1,185 (28.6)	26 (22.0)	55 (29.7)	71 (29.0)
High, No (%)	1,852 (39.5)	1,666 (40.9)	186 (30.5)	1,689 (40.8)	38 (32.2)	58 (31.4)	67 (27.4)
Lifestyle variables							
Smoking status§							
Never, No (%)	1,784 (37.8)	1,580 (38.6)	204 (33.0)	1,610 (38.7)	33 (27.5)	61 (32.6)	80 (32.1)
Former, No (%)	2,381 (50.5)	2,077 (50.7)	304 (49.2)	2,102 (50.5)	60 (50.0)	93 (49.7)	126 (50.6)
Current, No (%)	550 (11.7)	440 (10.7)	110 (17.8)	447 (10.8)	27 (22.5)	33 (17.7)	43 (17.3)
Alcohol use 							
None, No (%)	784 (16.6)	626 (15.3)	158 (25.6)	638 (15.3)	35 (29.2)	47 (25.1)	64 (25.8)
Low, No (%)	2,820 (59.8)	2,490 (60.8)	330 (53.5)	2,529 (60.8)	55 (45.8)	104 (55.6)	132 (53.2)
High, No (%)	1,110 (23.6)	981 (23.9)	129 (20.9)	992 (23.9)	30 (25.0)	36 (19.3)	52 (21.0)
Dutch Healthy Diet sum score#	84.4 (14.8)	84.8 (14.7)	81.6 (15.1)	84.7 (14.7)	81.6 (15.1)	82.3 (15.5)	80.7 (15.0)
Moderate to vigorous physical activity**, min/week	300 (180; 480)	315 (180; 495)	225 (105; 420)	315 (180; 495)	180 (90; 360)	270 (105; 450)	225 (135; 420)
Clinical characteristics							
Glucose metabolism status							
Normal glucose metabolism, No (%)	3,002 (63.3)	2,674 (64.9)	328 (52.7)	2,712 (64.8)	55 (45.5)	96 (50.8)	139 (55.8)
Prediabetes, No (%)	712 (15.0)	614 (14.9)	98 (15.8)	625 (14.9)	16 (12.3)	32 (16.9)	39 (15.7)
Type 2 diabetes, No (%)	1,030 (21.7)	834 (20.2)	196 (31.5)	848 (20.3)	50 (41.3)	61 (21.3)	71 (28.5)
	2,449 (51.7)	2,081 (50.5)	368 (59.2)	2,117 (50.6)	76 (62.8)	109 (57.7)	147 (59.0)
Prior cardiovascular disease**, No (%)	722 (15.4)	596 (14.6)	126 (20.6)	606 (14.6)	28 (23.5)	32 (17.2)	56 (22.8)
Body mass index‡‡, kg/m ²	26.7 (4.3)	26.5 (4.1)	28.0 (5.1)	26.5 (4.1)	28.9 (5.7)	27.6 (5.2)	28.0 (4.8)

Table S1. Characteristics of the total study population on flicker light-induced retinal dilation, and according to incident clinically relevant depressive symptoms and trajectories clinically relevant depressive symptoms (continued)

	Total study population (n=4,744)	According to clinically relevant depressive symptoms*		Trajectories of change of clinically relevant depressive symptoms†			
		No incident depressive symptoms (n=4,122, 86.9%)	Incident depressive symptoms (n=622, 13.1%)	Low (n=4,185, 88.2%)	Early-chronic (n=121, 2.6%)	Late-increasing (n=189, 4.0%)	Remitting (n=249, 5.3%)
Clinical characteristics							
Systolic blood pressure ^{††} , mmHg	133.2 (17.5)	133.1 (17.5)	133.8 (17.5)	133.1 (17.5)	136.3 (16.3)	133.3 (16.2)	133.3 (19.0)
Diastolic blood pressure ^{§§} , mmHg	75.6 (9.7)	75.5 (9.6)	76.0 (10.2)	75.5 (9.6)	78.1 (10.0)	74.8 (9.4)	75.9 (10.6)
Total-to-HDL cholesterol ratio ^{§§}	3.6 (1.2)	3.6 (1.2)	3.6 (1.2)	3.6 (1.2)	3.7 (1.3)	3.6 (1.2)	3.7 (1.1)
Lipid-modifying medication , No (%)	1,407 (29.7)	1,185 (28.8)	222 (35.7)	1,205 (28.8)	49 (39.7)	72 (38.1)	82 (32.9)
Antihypertensive medication , No (%)	1,664 (35.1)	1,385 (33.6)	279 (44.9)	1,411 (33.7)	64 (52.9)	83 (43.9)	106 (42.6)
Retinal microvascular measures							
Central retinal arteriolar caliber, μ m	138.9 (19.0)	138.9 (19.1)	138.8 (18.5)	138.9 (19.1)	136.4 (19.7)	138.8 (17.4)	140.3 (19.5)
Central retinal venular caliber, μ m	209.9 (29.2)	209.6 (29.1)	211.7 (29.9)	209.6 (29.1)	211.2 (29.3)	212.0 (30.6)	213.1 (30.1)
Flicker light-induced arteriolar dilation, measurement unit	4.37 (3.77)	4.43 (3.78)	3.96 (3.72)	4.24 (3.77)	3.89 (3.80)	4.42 (4.15)	3.64 (3.42)
Flicker light-induced venular dilation, measurement unit	7.51 (4.16)	7.54 (4.19)	7.31 (3.91)	7.52 (4.18)	7.94 (4.21)	7.39 (3.91)	7.07 (3.93)
Composite score of flicker light-induced retinal dilation, standard deviation	0.00 (1.00)	-0.01 (1.00)	0.10 (0.98)	-0.01 (1.00)	0.01 (1.03)	0.01 (1.03)	0.18 (0.93)
Baseline PHQ-9 score	2 (0; 4)	1 (0; 3)	4 (2; 6)	1 (0; 3)	6 (4; 8)	4 (2; 6)	4 (2; 6)

Data are means (standard deviation) or median (interquartile range).

* Clinically relevant depressive symptoms were defined as a PHQ-9 score of ≥ 10 ; † Graphical representation of trajectories of clinically relevant depressive symptoms is shown in Figure 2; ‡ data available in n=4,685; § data available in n=4,715; || data available in n=4,714; # data available in n=4,558; ** data available in n=4,749; †† data available in n=4,740; ††† data available in n=4,742; §§ data available in n=4,741 |||| data available in n=4,743.

HDL, high-density lipoprotein; PHQ-9, 9-item Patient Health Questionnaire.

Table S2. Characteristics of included study participants and excluded individuals

	Retinal microvascular calibers				Flicker light-induced retinal dilation			
	Included individuals (n=5,952)	Excluded individuals			Included individuals (n=4,744)	Excluded individuals		
		All† (n=1,737)	Excluded due to missing data (n=1,408)	Excluded to clinically relevant depressive symptoms at baseline (n=292)		All† (n=2,945)	Excluded due to missing data (n=2,616)	Excluded to clinically relevant depressive symptoms at baseline (n=292)
Demography								
Age, years	59.9 (8.5)	59.5 (9.2)	60.3 (9.2)	56.3 (8.4)	59.6 (8.5)	60.2 (8.9)	60.7 (8.9)	56.3 (8.4)
Sex, No (%)								
Female sex, No (%)	2,960 (49.7)	855 (49.2)	662 (47.0)	172 (58.9)	2,393 (50.4)	1,422 (48.3)	1,229 (47.0)	172 (58.9)
Male sex, No (%)	2,992 (50.3)	882 (50.8)	746 (53.0)	120 (41.1)	2,351 (49.6)	1,523 (51.7)	1,387 (53.0)	120 (41.1)
Education								
Low, No (%)	1,938 (33.0)	693 (40.9)	572 (41.5)	107 (37.7)	1,496 (31.9)	1,135 (39.3)	1,014 (39.4)	107 (37.7)
Intermediate, No (%)	1,633 (27.8)	459 (27.1)	354 (25.7)	95 (33.5)	1,337 (28.5)	755 (26.1)	650 (25.3)	95 (33.5)
High, No (%)	2,308 (39.3)	544 (32.1)	451 (32.8)	82 (28.9)	1,852 (39.5)	1,000 (34.6)	907 (35.3)	82 (28.9)
Lifestyle variables								
Smoking status								
Never, No (%)	2,249 (38.0)	586 (34.3)	478 (34.4)	97 (33.9)	1,784 (37.8)	1,051 (36.1)	943 (36.4)	97 (33.9)
Former, No (%)	2,969 (50.2)	795 (46.5)	649 (46.7)	129 (45.1)	2,381 (50.5)	1,383 (47.5)	1,237 (47.8)	129 (45.1)
Current, No (%)	697 (11.8)	330 (19.3)	262 (18.9)	60 (21.0)	550 (11.7)	477 (16.4)	409 (15.8)	60 (21.0)
Alcohol use								
None, No (%)	990 (16.7)	423 (24.7)	326 (23.5)	92 (32.2)	784 (16.6)	629 (21.6)	532 (20.6)	92 (32.2)
Low, No (%)	3,618 (59.5)	913 (53.4)	748 (53.9)	146 (51.1)	2,820 (59.8)	1,611 (55.3)	1,446 (55.9)	146 (51.1)
High, No (%)	1,407 (23.8)	374 (21.9)	314 (22.6)	48 (16.8)	1,110 (23.6)	671 (23.1)	611 (23.6)	48 (16.8)
Dutch Healthy Diet sum score	84.3 (15.0)	81.5 (15.3)	81.6 (15.2)	81.5 (15.7)	84.4 (14.8)	82.6 (15.6)	82.8 (15.6)	81.5 (15.7)
Moderate to vigorous physical activity, min/week	285 (180; 480)	270 (135; 480)	270 (90; 450)	180 (60; 360)	300 (180; 480)	270 (135; 450)	240 (105; 450)	180 (60; 360)
Clinical characteristics								
Glucose metabolism status								
Normal glucose metabolism, No (%)	3,723 (62.6)	882 (50.7)	727 (51.6)	155 (53.1)	3,002 (63.3)	1,603 (54.4)	1,448 (55.4)	155 (53.1)
Prediabetes, No (%)	894 (15.0)	247 (14.2)	211 (15.0)	36 (12.3)	712 (15.0)	429 (14.5)	393 (15.0)	36 (12.3)
Type 2 diabetes, No (%)	1,335 (22.4)	558 (32.1)	459 (32.6)	99 (33.9)	1,030 (21.7)	863 (29.3)	764 (29.2)	99 (33.9)
Other types of diabetes, No (%)	0 (0)	50 (2.9)	11 (0.8)	2 (0.7)	0 (0)	50 (1.7)	11 (0.4)	2 (0.7)
	3,147 (52.9)	1,035 (59.6)	833 (59.2)	180 (61.6)	2,449 (51.7)	1,733 (58.9)	1,531 (58.6)	180 (61.6)
Prior cardiovascular disease, No (%)	960 (16.3)	339 (20.0)	270 (19.6)	64 (22.6)	722 (15.4)	577 (19.9)	508 (19.7)	64 (22.6)
Body mass index, kg/m ²	26.8 (4.4)	27.7 (5.0)	27.6 (4.9)	28.5 (5.5)	26.7 (4.3)	27.4 (4.8)	27.4 (4.7)	28.5 (5.5)
Systolic blood pressure, mmHg	133.4 (17.7)	135.1 (18.7)	135.5 (19.0)	133.2 (16.7)	133.2 (17.5)	134.7 (18.5)	134.9 (18.7)	133.2 (16.7)
Diastolic blood pressure, mmHg	75.5 (9.7)	75.8 (10.1)	75.8 (10.0)	76.3 (10.1)	75.6 (9.7)	75.5 (10.0)	75.5 (10.0)	76.3 (10.1)

Table S2. Characteristics of included study participants and excluded individuals (continued)

	Retinal microvascular calibers				Flicker light-induced retinal dilation			
	Included individuals (n=5,952)	Excluded individuals			Included individuals (n=4,744)	Excluded individuals		
		All* (n=1,737)	Excluded due to missing data (n=1,408)	Excluded to clinically relevant depressive symptoms at baseline (n=292)		All† (n=2,945)	Excluded due to missing data (n=2,616)	Excluded to clinically relevant depressive symptoms at baseline (n=292)
Clinical characteristics (continued)								
Total-to-HDL cholesterol ratio	3.6 (1.2)	3.8 (1.3)	3.8 (1.3)	3.8 (1.4)	3.6 (1.2)	3.7 (1.2)	3.7 (1.2)	3.8 (1.4)
Lipid-modifying medication, No (%)	1,818 (30.6)	686 (39.5)	549 (39.1)	113 (38.7)	1,407 (29.7)	1,097 (37.3)	960 (36.8)	113 (38.7)
Antihypertensive medication, No (%)	2,178 (36.6)	758 (43.7)	600 (42.7)	140 (48.0)	1,664 (35.1)	1,272 (43.3)	1,114 (42.7)	140 (48.0)
Retinal microvascular measures								
Central retinal arteriolar caliber, μm	138.1 (19.7)	140.2 (19.9)	139.8 (20.0)	141.1 (19.3)	138.9 (19.0)	137.5 (20.9)	137.0 (21.1)	141.1 (19.3)
Central retinal venular caliber, μm	208.7 (30.1)	212.1 (30.6)	211.8 (31.1)	213.1 (28.9)	209.9 (29.2)	207.7 (31.8)	207.0 (32.1)	213.1 (28.9)
Flicker light-induced arteriolar dilation, measurement unit	4.39 (3.75)	3.97 (3.77)	3.99 (3.83)	4.09 (3.69)	4.37 (3.77)	4.03 (3.68)	4.08 (3.72)	4.09 (3.69)
Flicker light-induced venular dilation, measurement unit	7.51 (4.17)	7.23 (4.12)	7.33 (4.08)	7.06 (4.28)	7.51 (4.16)	7.26 (4.20)	7.37 (4.19)	7.06 (4.28)
Baseline PHQ-9 score	2 (0; 4)	3 (1; 10)	2 (0; 5)	12 (11; 16)	2 (0; 4)	2 (0; 6)	2 (0; 4)	12 (11; 16)

Data are means (standard deviation) or median (interquartile range).

* Individuals were excluded due missing data on depressive symptoms at baseline or follow-up (n=834), clinically relevant depressive symptoms at baseline (n=292), to other types of diabetes than type 2 (n=37), or missing data on retinal microvascular calibers (n=574); † Individuals were missing data on depressive symptoms at baseline or follow-up (n=834), clinically relevant depressive symptoms at baseline (n=292), to other types of diabetes than type 2 (n=37), or missing data on flicker light-induced retinal dilation (n=1,782).
HDL, high-density lipoprotein; PHQ-9, 9-item Patient Health Questionnaire.

Table S3. Estimation of trajectories of presence of clinically relevant depressive symptoms (PHQ-9 ≥ 10): model fit statistics (group based trajectory models)

Number of groups*	Trajectory shape†	Allocated Group membership‡	BIC§ (n=6,563 participants)	BIC§ (n=44,356 observations)	Average Posterior Probabilities
1	2	6,563 (100)	-6811.38	-6814.25	N/A
2	2	6,031 (91.9)	-5693.84	-5700.53	0.97
	2	532 (8.1)			0.88
3	2	215 (3.3)	-5625.49	-5614.98	0.74
	2	5,911 (90.1)			0.95
	2	437 (6.7)			0.84
4	2	72 (1.1)	-5569.84	-5584.17	0.69
	2	475 (7.2)			0.85
	2	5,797 (88.3)			0.91
	2	219 (3.3)			0.73
4#	1	276 (4.2)	-5579.97	-5593.35	0.68
	2	5,755 (87.7)			0.92
	2	354 (5.4)			0.71
	2	178 (2.7)			0.81
4	1	332 (5.1)	-5585.07	-5597.49	0.67
	1	5,868 (86.6)			0.93
	2	375 (5.7)			0.63
	2	170 (2.6)			0.82
4	1	668 (10.2)	-5637.85	-5649.32	0.80
	1	5,665 (86.3)			0.86
	1	39 (0.6)			0.58
	2	191 (2.9)			0.81
4	1	160 (2.4)	-5662.10	-5672.61	0.80
	1	5,665 (86.3)			0.89
	1	695 (10.5)			0.81
	1	43 (0.7)			0.75
5	2	384 (5.6)	-5574.24	-5592.39	0.63
	2	5,821 (88.7)			0.93
	2	148 (2.3)			0.61
	2	183 (2.8)			0.63
	2	27 (0.4)			0.80

* Number of trajectory groups estimated.

† Polynomial function of time (1 linear, 2 quadratic).

‡ Trajectories were fitted among all individuals without clinically relevant depressive symptoms at baseline, with available data on depressive symptoms at baseline and on one or more follow-up examinations (n=6,563, Figure S1).

§ Bayesian Information Criterion (BIC), a difference of 10 is strong evidence that the model with the lowest BIC (compared to null) has best fit.

|| Posterior probabilities of group membership for individuals assigned to each group.

Model was selected based on number of individuals per group (n>100), BIC (difference with previous model ~10), posterior probabilities of group membership ~ 0.70 and clinical interpretation.

Table S4. Interactions with age, sex, blood pressure and glucose metabolism status for the association of between central retinal arteriolar caliber (CRAE), central retinal venular caliber (CRVE) and the composite score of flicker light-induced retinal dilation and incident clinically relevant depressive symptoms (PHQ-9 score ≥ 10)

		Incident clinically relevant depressive symptoms
		P for interaction
Interaction with age (continuous scale)		
CRAE (SD)		0.711
CRVE (SD)		0.940
Composite score of flicker light-induced retinal dilation (SD)		0.019*
Interaction with sex (female vs male)		
CRAE (SD)		0.054
CRVE (SD)		0.051
Composite score of flicker light-induced retinal dilation (SD)		0.683
Interaction with systolic blood pressure (continuous scale)		
CRAE (SD)		0.759
CRVE (SD)		0.317
Composite score of flicker light-induced retinal dilation (SD)		0.297
Interaction with glucose metabolism status		
CRAE (SD)	Prediabetes vs normal glucose metabolism	0.206
	Type 2 diabetes vs normal glucose metabolism	0.389
CRVE (SD)	Prediabetes vs normal glucose metabolism	0.296
	Type 2 diabetes vs normal glucose metabolism	0.103
Composite score of flicker light-induced retinal dilation (SD)	Prediabetes vs normal glucose metabolism	0.231
	Type 2 diabetes vs normal glucose metabolism	0.801

All analyses adjusted for age, sex, education, glucose metabolism status, body mass index, smoking, alcohol use, total/HDL ratio, lipid-modifying medication use, dietary habits and moderate to vigorous physical activity, prior cardiovascular disease, systolic blood pressure, antihypertensive medication use and baseline PHQ-9 score.

* Association was stronger in younger individuals compared to older individuals.

SD, standard deviation; PHQ-9, 9-item patient health questionnaire; HDL, high-density lipoprotein.

Table S5. Interactions with age, sex, blood pressure and glucose metabolism status for the association of between central retinal arteriolar caliber (CRAE), central retinal venular caliber (CRVE) and the composite score of flicker light-induced retinal dilation and trajectories of clinically relevant depressive symptoms* (PHQ-9 score ≥ 10)

		Retinal microvascular calibers		Composite score of flicker light-induced retinal dilation (SD)
		CRAE (SD)	CRVE (SD)	
		P for interaction		
Interaction with age (continuous scale)				
Low		Reference	Reference	Reference
Early-chronic		0.328	0.181	0.359
Late-increasing		0.158	0.937	0.236
Remitting		0.838	0.447	0.139
Interaction with sex (female vs male)				
Low		Reference	Reference	Reference
Early-chronic		0.143	0.192	0.627
Late-increasing		0.886	0.991	0.266
Remitting		0.016 [†]	0.112	0.884
Interaction with systolic blood pressure (continuous scale)				
Low		Reference	Reference	Reference
Early-chronic		0.267	0.002 [‡]	0.089
Late-increasing		0.184	0.234	0.523
Remitting		0.556	0.627	0.607
Interaction with glucose metabolism status				
Low	Prediabetes vs normal glucose metabolism	Reference	Reference	Reference
	Type 2 diabetes vs normal glucose metabolism	Reference	Reference	Reference
Early-chronic	Prediabetes vs normal glucose metabolism	0.035 [§]	0.191	0.204
	Type 2 diabetes vs normal glucose metabolism	0.836	0.395	0.291
Late-increasing	Prediabetes vs normal glucose metabolism	0.743	0.401	0.375
	Type 2 diabetes vs normal glucose metabolism	0.388	0.658	0.250
Remitting	Prediabetes vs normal glucose metabolism	0.790	0.510	0.994
	Type 2 diabetes vs normal glucose metabolism	0.201	0.034	0.413

All analyses adjusted for age, sex, education, glucose metabolism status, body mass index, smoking, alcohol use, total/HDL ratio, lipid-modifying medication use, dietary habits and moderate to vigorous physical activity, prior cardiovascular disease, systolic blood pressure, antihypertensive medication use and baseline PHQ-9 score. * Graphical representation of trajectories of clinically relevant depressive symptoms is shown in Figure 2. [†] Association was stronger in women compared to men. [‡] Association was stronger in those without hypertension. [§] Association was stronger in those with prediabetes compared to those with normal glucose metabolism. ^{||} Association was stronger in those with type 2 diabetes compared to those with normal glucose metabolism status. SD, standard deviation; PHQ-9, 9-item patient health questionnaire; HDL, high-density lipoprotein.

Table S6. Association between flicker light-induced retinal arteriolar and venular dilation and trajectories of clinically relevant depressive symptoms* (PHQ-9 score ≥ 10)

		Flicker light-induced retinal dilation	
		Flicker light-induced retinal arteriolar dilation (SD)	Flicker light-induced retinal venular dilation (SD)
		Odds ratio (95% Confidence Interval)	
Trajectories	n	Model 1	
Low	4,185	Reference	Reference
Early-chronic	121	1.13 (0.93; 1.37)	0.91 (0.76; 1.08)
Late-increasing	189	0.97 (0.84; 1.12)	1.03 (0.89; 1.20)
Remitting	249	1.26 (1.09; 1.46)	1.14 (0.99; 1.31)
		Model 2	
Low	4,185	Reference	Reference
Early-chronic	121	1.12 (0.92; 1.36)	0.92 (0.77; 1.09)
Late-increasing	189	0.96 (0.83; 1.11)	1.03 (0.89; 1.20)
Remitting	249	1.26 (1.09; 1.46)	1.15 (1.00; 1.32)
		Model 3	
Low	4,185	Reference	Reference
Early-chronic	121	1.11 (0.91; 1.35)	0.91 (0.76; 1.08)
Late-increasing	189	0.96 (0.83; 1.11)	1.03 (0.89; 1.20)
Remitting	249	1.25 (1.09; 1.45)	1.14 (0.99; 1.31)
		Model 4	
Low	4,185	Reference	Reference
Early-chronic	121	1.10 (0.90; 1.35)	0.90 (0.75; 1.09)
Late-increasing	189	0.95 (0.82; 1.10)	1.02 (0.87; 1.18)
Remitting	249	1.24 (1.07; 1.44)	1.13 (0.98; 1.30)

Results are reported for flicker light induced retinal arteriolar and venular dilation per one lower standard deviation. Model 1 adjusted for age, sex, education and glucose metabolism status. Model 2 additionally adjusted for body mass index, smoking, alcohol use, total/HDL ratio, lipid-modifying medication use, dietary habits and moderate to vigorous physical activity. Model 3 additionally adjusted for prior cardiovascular disease, systolic blood pressure and antihypertensive medication use. Model 4 additionally adjusted for baseline PHQ-9 score.

* Graphical representation of trajectories of clinically relevant depressive symptoms is shown in Figure 2. SD, standard deviation; PHQ-9, 9-item patient health questionnaire; HDL, high-density lipoprotein.

Table S7. Association between central retinal arteriolar caliber (CRAE), central retinal venular caliber (CRVE) and the composite score of flicker light-induced retinal dilation and incident clinically relevant depressive symptoms (PHQ-9 score ≥ 10) – additional analyses

	Incident clinically relevant depressive symptoms
	Hazard ratio (95% Confidence interval)
Excluding individuals with a major depression at baseline*	
CRAE (SD)	0.89 (0.83; 0.96)
CRVE (SD)	0.92 (0.86; 0.99)
Composite score of flicker light-induced retinal dilation (SD)	1.11 (1.02; 1.21)
Excluding individuals using antidepressant medication at baseline†	
CRAE (SD)	0.89 (0.82; 0.96)
CRVE (SD)	0.93 (0.86; 1.00)
Composite score of flicker light-induced retinal dilation (SD)	1.10 (1.00; 1.20)
Excluding individuals with a lifetime history of depression‡	
CRAE (SD)	0.84 (0.76; 0.93)
CRVE (SD)	0.88 (0.80; 0.98)
Composite score of flicker light-induced retinal dilation (SD)	1.11 (0.99; 1.24)
Adjustment for income instead of education	
CRAE (SD)	0.89 (0.83; 0.96)
CRVE (SD)	0.93 (0.86; 0.99)
Composite score of flicker light-induced retinal dilation (SD)	1.10 (1.01; 1.19)
Adjustment for 24 hour systolic blood pressure instead of office systolic blood pressure	
CRAE (SD)	0.90 (0.83; 0.96)
CRVE (SD)	0.93 (0.86; 0.99)
Composite score of flicker light-induced retinal dilation (SD)	1.11 (1.01; 1.20)
Adjustment for waist circumference instead of body mass index	
CRAE (SD)	0.89 (0.83; 0.96)
CRVE (SD)	0.93 (0.86; 0.99)
Composite score of flicker light-induced retinal dilation (SD)	1.10 (1.01; 1.20)
Adjustment for moderate-to-vigorous physical activity measured by an accelerometer instead of by questionnaire	
CRAE (SD)	0.89 (0.83; 0.96)
CRVE (SD)	0.93 (0.86; 0.99)
Composite score of flicker light-induced retinal dilation (SD)	1.11 (1.02; 1.20)
Additional adjustment for markers of low-grade inflammation	
CRAE (SD)	0.89 (0.83; 0.95)
CRVE (SD)	0.92 (0.86; 0.99)
Composite score of flicker light-induced retinal dilation (SD)	1.10 (1.01; 1.20)
Flicker light-induced retinal arteriolar and venular dilation as average percentage dilation over baseline diameter	
CRAE (SD)	n/a
CRVE (SD)	n/a
Composite score of flicker light-induced retinal dilation (SD)	1.06 (0.97; 1.15)
Mutual adjustment for CRVE or CRAE	
CRAE (SD)	0.88 (0.79; 0.96)
CRVE (SD)	1.02 (0.92; 1.13)
Composite score of flicker light-induced retinal dilation (SD)	n/a
Complete case analysis§	
CRAE (SD)	0.86 (0.80; 0.93)
CRVE (SD)	0.90 (0.83; 0.97)
Composite score of flicker light-induced retinal dilation (SD)	1.09 (1.00; 1.20)

All analyses adjusted for age, sex, education, glucose metabolism status, body mass index, smoking, alcohol use, total/HDL ratio, lipid-modifying medication use, dietary habits and moderate to vigorous physical activity, prior cardiovascular disease, systolic blood pressure, antihypertensive medication use and baseline PHQ-9 score. * n=85 and n=73 had a major depression at

baseline according to the MINI-International Neuropsychiatric Interview (Sheehan et al., 1998) in the analysis on retinal microvascular calibers and flicker light-induced retinal dilation, respectively. [†] n=341 and n=267 used antidepressant medication at baseline in the analysis on retinal microvascular calibers and flicker light-induced retinal dilation, respectively. [‡] n=1,622 and n=1,313 had a lifetime history depression according to the MINI-International Neuropsychiatric Interview (Sheehan et al., 1998) in the analysis on retinal microvascular calibers and flicker light-induced retinal dilation, respectively. [§] n=5,289 and n=4,238 in the analysis on retinal microvascular calibers and flicker light-induced retinal dilation, respectively. SD, standard deviation; PHQ-9, 9-item patient health questionnaire; HDL, high-density lipoprotein.

Table S8. Association between central retinal arteriolar caliber (CRAE), central retinal venular caliber (CRVE) and the composite score of flicker light-induced retinal dilation and trajectories of clinically relevant depressive symptoms* – additional analyses

	Retinal microvascular calibers		Composite score of flicker-light induced retinal dilation (SD)
	CRAE (SD)	CRVE (SD)	
Trajectories	Odds ratio (95% Confidence interval)		
Excluding individuals with a major depression at baseline[†]			
Low	Reference	Reference	Reference
Early-chronic	0.78 (0.64; 0.94)	0.88 (0.74; 1.05)	1.05 (0.84; 1.30)
Late-increasing	0.96 (0.84; 1.10)	0.99 (0.87; 1.13)	0.92 (0.79; 1.07)
Remitting	0.93 (0.82; 1.05)	0.96 (0.85; 1.08)	1.18 (1.02; 1.36)
Excluding individuals using antidepressant medication at baseline[‡]			
Low	Reference	Reference	Reference
Early-chronic	0.83 (0.68; 1.01)	0.92 (0.76; 1.11)	0.99 (0.80; 1.23)
Late-increasing	0.96 (0.83; 1.10)	1.00 (0.88; 1.15)	1.01 (0.87; 1.19)
Remitting	0.91 (0.80; 1.03)	0.97 (0.85; 1.10)	1.19 (1.01; 1.39)
Excluding individuals with a lifetime history of depression[§]			
Low	Reference	Reference	Reference
Early-chronic	0.81 (0.62; 1.05)	0.99 (0.76; 1.30)	1.05 (0.77; 1.42)
Late-increasing	0.86 (0.71; 1.03)	0.88 (0.74; 1.06)	1.01 (0.82; 1.24)
Remitting	0.89 (0.75; 1.04)	0.92 (0.78; 1.08)	1.17 (0.96; 1.42)
Adjustment for income instead of education			
Low	Reference	Reference	Reference
Early-chronic	0.82 (0.69; 0.98)	0.92 (0.77; 1.09)	0.98 (0.81; 1.19)
Late-increasing	0.94 (0.82; 1.08)	0.98 (0.86; 1.12)	0.98 (0.85; 1.14)
Remitting	0.92 (0.81; 1.04)	0.97 (0.86; 1.09)	1.23 (1.06; 1.42)
Adjustment for 24 hour systolic blood pressure instead of office systolic blood pressure			
Low	Reference	Reference	Reference
Early-chronic	0.83 (0.69; 0.99)	0.92 (0.77; 1.09)	0.99 (0.81; 1.21)
Late-increasing	0.96 (0.84; 1.10)	0.99 (0.87; 1.13)	0.98 (0.84; 1.13)
Remitting	0.92 (0.81; 1.04)	0.97 (0.86; 1.09)	1.23 (1.07; 1.43)
Adjustment for waist circumference instead of body mass index			
Low	Reference	Reference	Reference
Early-chronic	0.83 (0.69; 0.99)	0.93 (0.78; 1.09)	0.99 (0.81; 1.21)
Late-increasing	0.94 (0.82; 1.08)	0.98 (0.86; 1.12)	0.98 (0.84; 1.13)
Remitting	0.92 (0.81; 1.04)	0.97 (0.86; 1.09)	1.23 (1.06; 1.42)

Table S8. Association between central retinal arteriolar caliber (CRAE), central retinal venular caliber (CRVE) and the composite score of flicker light-induced retinal dilation and trajectories of clinically relevant depressive symptoms* – additional analyses (continued)

	Retinal microvascular calibers		Composite score of flicker-light induced retinal dilation (SD)
	CRAE (SD)	CRVE (SD)	
Trajectories	Odds ratio (95% Confidence interval)		
Adjustment for moderate-to-vigorous physical activity measured by an accelerometer instead of by questionnaire			
Low	Reference	Reference	Reference
Early-chronic	0.83 (0.69; 0.99)	0.92 (0.78; 1.09)	1.00 (0.82; 1.22)
Late-increasing	0.94 (0.83; 1.08)	0.98 (0.86; 1.12)	0.98 (0.84; 1.14)
Remitting	0.92 (0.81; 1.04)	0.97 (0.86; 1.09)	1.24 (1.07; 1.44)
Additional adjustment for markers of low-grade inflammation			
Low	Reference	Reference	Reference
Early-chronic	0.82 (0.69; 0.98)	0.92 (0.77; 1.09)	0.99 (0.81; 1.21)
Late-increasing	0.94 (0.82; 1.08)	0.98 (0.86; 1.12)	0.98 (0.84; 1.13)
Remitting	0.92 (0.81; 1.03)	0.97 (0.86; 1.09)	1.23 (1.06; 1.43)
Flicker light-induced retinal arteriolar and venular dilation as average percentage dilation over baseline diameter			
Low	n/a	n/a	Reference
Early-chronic	n/a	n/a	1.01 (0.82; 1.23)
Late-increasing	n/a	n/a	0.91 (0.78; 1.05)
Remitting	n/a	n/a	1.18 (1.02; 1.37)
Mutual adjustment for CRVE or CRAE			
Low	Reference	Reference	n/a
Early-chronic	0.75 (0.57; 0.98)	1.13 (0.87; 1.46)	n/a
Late-increasing	0.90 (0.74; 1.11)	1.06 (0.87; 1.29)	n/a
Remitting	0.87 (0.73; 1.05)	1.07 (0.90; 1.27)	n/a
Complete case analysis^{ll}			
Low	Reference	Reference	Reference
Early-chronic	0.80 (0.66; 0.98)	0.92 (0.76; 1.11)	0.98 (0.79; 1.21)
Late-increasing	0.91 (0.78; 1.05)	0.93 (0.81; 1.07)	0.98 (0.83; 1.15)
Remitting	0.90 (0.79; 1.03)	0.94 (0.83; 1.07)	1.25 (1.07; 1.46)

All analyses adjusted for age, sex, education, glucose metabolism status, body mass index, smoking, alcohol use, total/HDL ratio, lipid-modifying medication use, dietary habits and moderate to vigorous physical activity, prior cardiovascular disease, systolic blood pressure, antihypertensive medication use and baseline PHQ-9 score. * Graphical representation of trajectories of clinically relevant depressive symptoms is shown in Figure 2. † n=85 and n=73 had a major depression at baseline according to the MINI-International Neuropsychiatric Interview (Sheehan et al., 1998) in the analysis on retinal microvascular calibers and flicker light-induced retinal dilation, respectively. ‡ n=341 and n=267 used antidepressant medication at baseline in the analysis on retinal microvascular calibers and flicker light-induced retinal dilation, respectively. § n=1,622 and n=1,313 had a lifetime history depression according to the MINI-International Neuropsychiatric Interview (Sheehan et al., 1998) in the analysis on retinal microvascular calibers and flicker light-induced retinal dilation, respectively. || n=5,289 and n=4,238 in the analysis on retinal microvascular calibers and flicker light-induced retinal dilation, respectively. SD, standard deviation; PHQ-9, 9-item patient health questionnaire; HDL, high-density lipoprotein.

Table S9. Association between prior cardiovascular disease and incident clinically relevant depressive symptoms (PHQ-9 score ≥ 10) with and without adjustment for central retinal arteriolar caliber (CRAE), central retinal venular caliber (CRVE) and the composite score of flicker light-induced retinal dilation

	Incident clinically relevant depressive symptoms
	Hazard ratio (95% Confidence interval)
Study population on retinal microvascular calibers	
Model 1	1.30 (1.09; 1.56)
Model 2	1.24 (1.03; 1.49)
Model 3	1.21 (1.01; 1.46)
Model 4	1.12 (0.93; 1.35)
Model 3 + adjustment for CRAE	1.21 (1.00; 1.46)
Model 3 + adjustment for CRVE	1.21 (1.00; 1.46)
Model 4 + adjustment for CRAE	1.11 (0.92; 1.33)
Model 4 + adjustment for CRVE	1.11 (0.92; 1.34)
Study population on flicker light-induced retinal dilation	
Model 1	1.42 (1.16; 1.73)
Model 2	1.38 (1.13; 1.71)
Model 3	1.35 (1.09; 1.67)
Model 4	1.26 (1.02; 1.56)
Model 3 + adjustment for composite score of flicker light-induced retinal dilation	1.35 (1.09; 1.66)
Model 4 + adjustment for composite score of flicker light-induced retinal dilation	1.26 (1.02; 1.55)

Results are reported as differences in risk for incident clinically relevant depressive symptoms in those with prior cardiovascular disease compared to those without. Model 1 adjusted for age, sex, education and glucose metabolism status. Model 2 additionally adjusted for body mass index, smoking, alcohol use, total/HDL ratio, lipid-modifying medication use, dietary habits and moderate to vigorous physical activity. Model 3 additionally adjusted for systolic blood pressure and antihypertensive medication use. Model 4 additionally adjusted for baseline PHQ-9 score. SD, standard deviation; PHQ-9, 9-item patient health questionnaire; HDL, high-density lipoprotein.

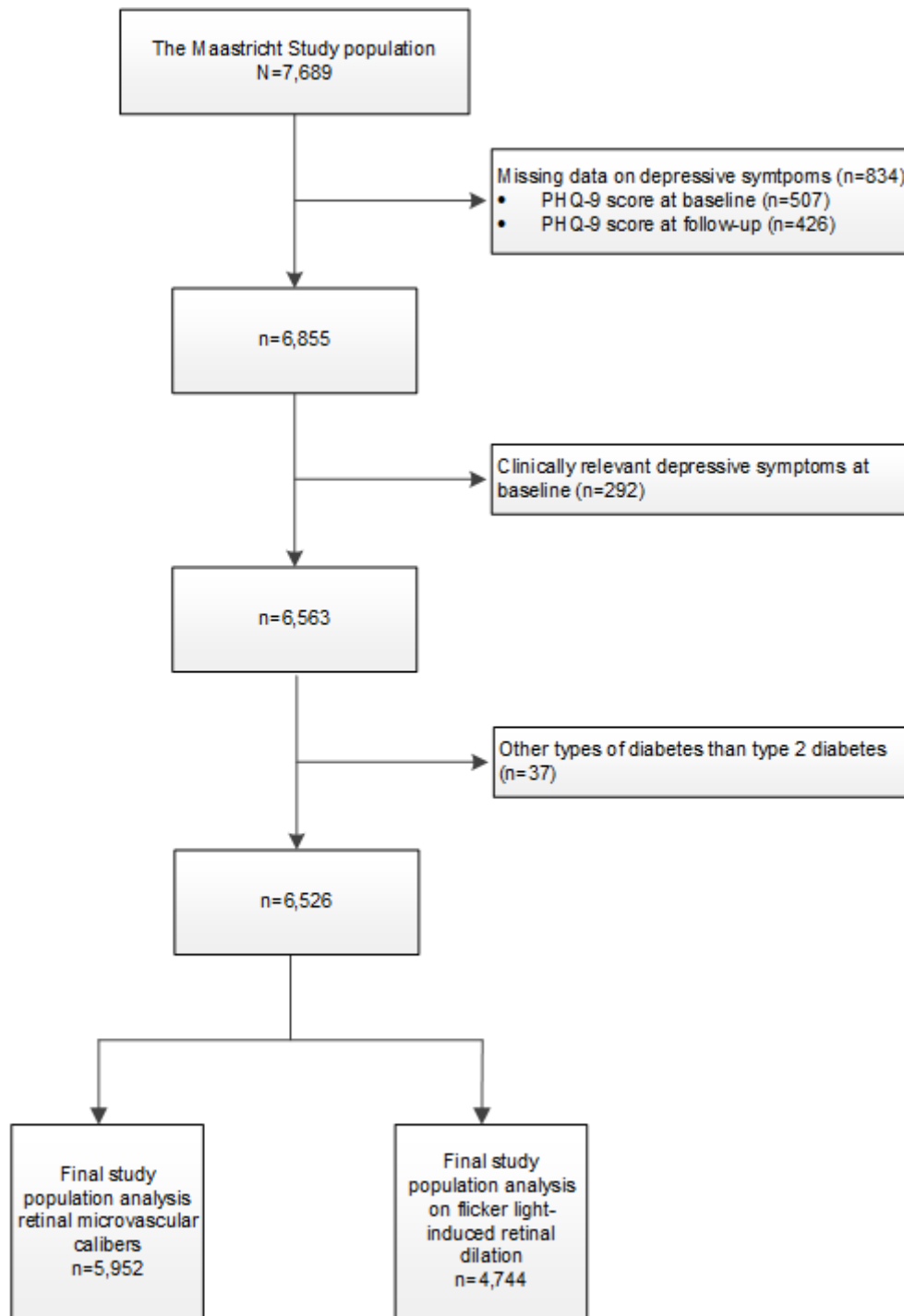


Figure S1. Flowchart derivation of the study populations

Numbers not mutually exclusive. Of the 5,952 participants with data on retinal microvascular calibers, n=5,601, n=5,124, n=4,939, n=4,617, n=4,528, n=3,789, n=3,043, n=1,922 and n=570 had data on depressive symptoms at the first, second, third, fourth, fifth, sixth, seventh and ninth follow-up examination, respectively. Of the 4,744 participants with data on flicker light-induced retinal dilation, n=4,476, n=4,098, n=3,952, n=3,712, n=3,668, n=3,075, n=2,497, n=1,672 and n=525 had data on depressive symptoms at the first, second, third, fourth, fifth, sixth, seventh and ninth follow-up examination, respectively.

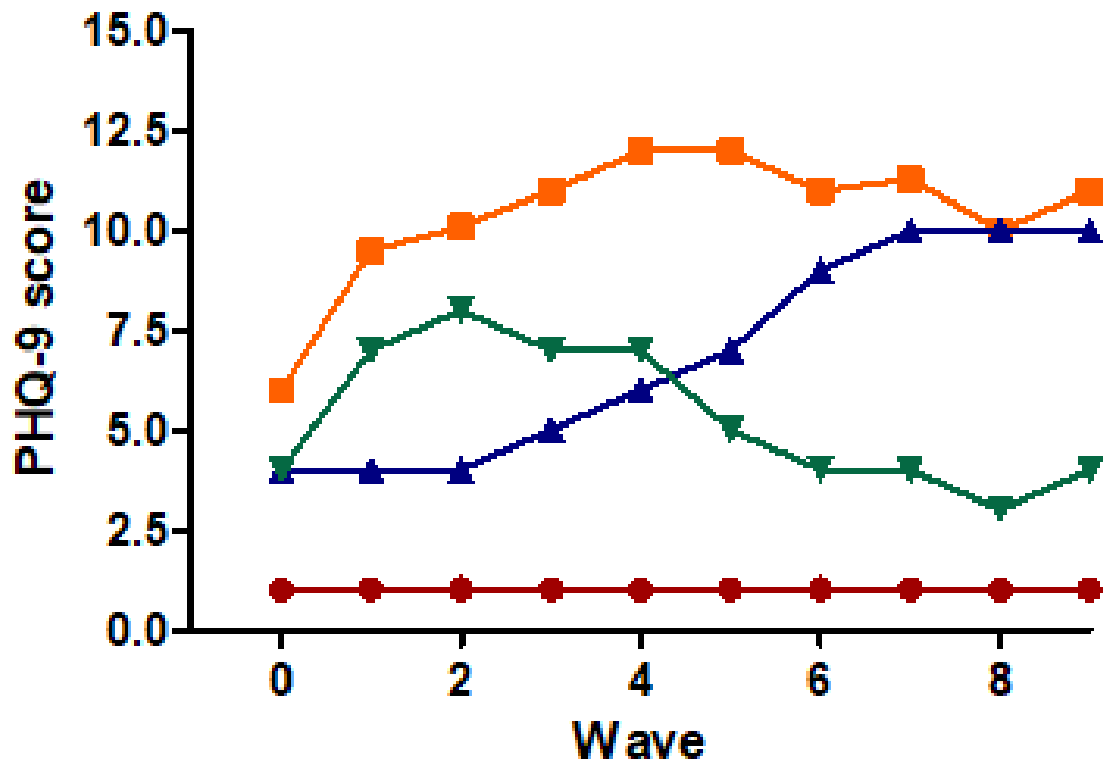


Figure S2. Median PHQ-9 score by wave in each trajectory of clinically relevant depressive symptoms* (PHQ-9 score ≥ 10)

* Graphical representation of trajectories of clinically relevant depressive symptoms is shown in Figure 2. PHQ-9, 9-item patient health questionnaire.

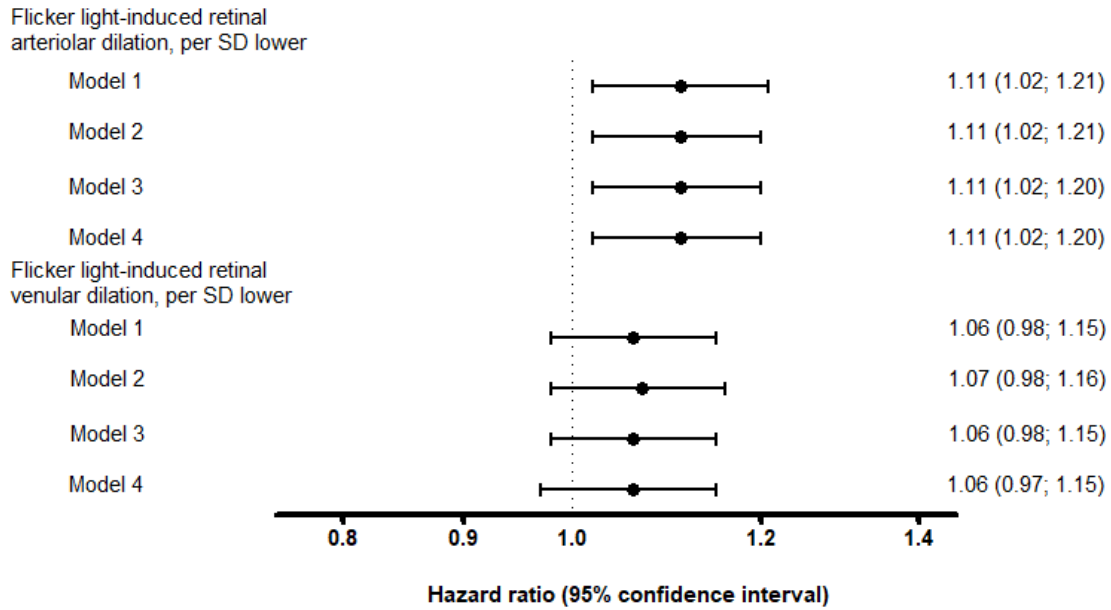


Figure S3. Association between flicker light-induced retinal arteriolar and venular dilation and incident clinically relevant depressive symptoms (PHQ-9 score ≥ 10).

Results are reported for flicker light induced retinal arteriolar and venular dilation per one lower standard deviation. Model 1 adjusted for age, sex, education and glucose metabolism status. Model 2 additionally adjusted for body mass index, smoking, alcohol use, total/HDL ratio, lipid-modifying medication use, dietary habits and moderate to vigorous physical activity. Model 3 additionally adjusted for prior cardiovascular disease, systolic blood pressure and antihypertensive medication use. Model 4 additionally adjusted for baseline PHQ-9 score.

SD, standard deviation; PHQ-9, 9-item patient health questionnaire; HDL, high-density lipoprotein.

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