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Supplemental information

Plaque characteristics and biomarkers predicting regression and progression of carotid atherosclerosis

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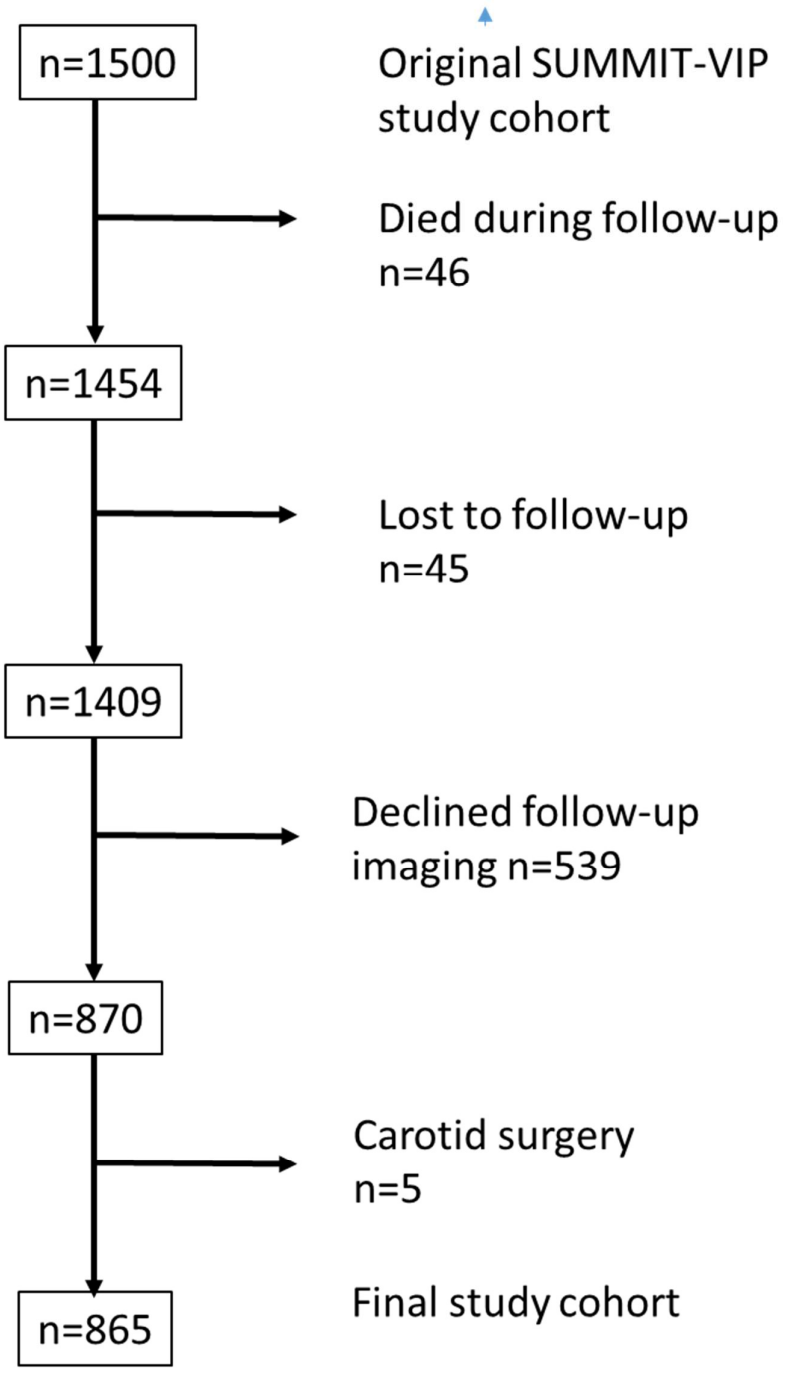
On-line supplemental data



Supplemental figure 1. Consort flow diagram of study cohort. Related to table 1

Supplemental table 1. Baseline clinical characteristics of study subjects with and without T2D. Related to table 1.

	T2D (n=566)	Non-T2D (n=299)	P
Age (years)	68.5±8.0	67.4±9.1	0.07
Gender (% males)	70.0	59.4	0.09
Current smokers (%)	8.7	6.4	0.30
Prevalent CVD (%)	39.2	41.3	0.56
Duration of T2D (years)	10.7±7.8	-	
BMI (kg/m^{-2})	30.3±5.0	26.9±4.0	<0.001
Medications			
Insulin (%)	25.4	0	
Statin (%)	74.3	49.8	<0.001
ACE inhibitors (%)	43.6	24.7	<0.001
Metformin (%)	70.0	0	
Betablockers (%)	34.5	26.6	0.02
Metabolic factors			
HbA1c (mmol/mmol)	57.1±13.2	38.9±3.9	<0.001
LDL (mmol/L)	2.22±0.88	2.81±0.90	<0.001
HDL (mmol/L)	1.28±0.39	1.52±0.42	<0.001
Triglycerides (mmol/L)	1.40 (1.00-2.09)	1.11 (0.83-1.54)	<0.001
Blood pressure			
Systolic (mmHg)	136±18	132±17	0.006
Diastolic (mmHg)	76±10	76±9	0.94
Renal function			
eGFR (mL/min^{-1} per $1.73m^2$)	79.8±20.3	80.8±17.0	0.47

Variables with normal distribution are shown as mean±standard deviation and skewed variables as median and interquartile range. eGFR; estimated glomerular filtration rate. Differences between means of normally distributed continuous variables were assessed with independent sample *t* tests and between skewed variables with the Mann-Whitney U-test. χ^2 test was used for categorical variables.

Supplemental table 2. Relationships between medications and change in carotid IMT. Related to table 2.

	Mean IMT			
	Regression	No change	Progression	P for trend
CCA	(n=181)	(n=379)	(n=299)	
Statin (%)	69.1	66.4	63.5	ns
ACE inhibitors (%)	36.1	39.0	35.1	ns
Beta-blockers (%)	37.8	30.0	30.9	ns
Antiplatelet (%)	44.8	47.1	45.3	ns
Bulb	(n=192)	(n=206)	(n=412)	
Statin (%)	67.4	60.3	65.0	ns
ACE inhibitors (%)	34.2	33.3	38.1	ns
Beta-blockers (%)	29.6	26.1	33.2	ns
Antiplatelet (%)	45.5	39.2	43.3	ns

P for trend was calculated by Chi-square linear-by-linear association.

Supplemental table 3. Relationships between baseline clinical characteristics with change in carotid IMT using ±0.1 mm as cut-off for no change in IMT. Related to table 2.

	Mean bulb IMT		
	Regression n=137	No change n=367	Progression n=302
Age (years)	68.2±8.4	67.0±8.6	69.5±8.1***
Male gender (%)	54.0	61.9	69.9*
Current smokers (%)	6.9	7.4	7.5
BMI (kg/m^{-2})	29.1±4.9*	28.1±4.8	28.8±4.5
Metabolic factors			
HbA1c (mmol/mmol)	48.8 (40.8-60.0)	46.0 (40.0-57.0)	48.0 (39.0-59.0)
LDL (mmol/L)	2.23±0.94**	2.48±0.92	2.53±0.92
HDL (mmol/L)	1.34±0.40	1.40±0.43	1.36±0.42
TG (mmol/L)	1.48 (1.04-2.04)	1.20 (0.90-1.67)	1.30 (0.91-1.89)
Blood pressure			
Systolic (mmHg)	138±17*	132±17	137±17**
Diastolic (mmHg)	77±9	76±9	76±10
Renal function			
eGFR	78.5±20.9	82.2±17.5	79.0±20.4
	Mean CCA IMT		
	Regression n=88	No change N=633	Progression n=142
Age (years)	68.2±8.4	68.1±8.5	68.1±8.2
Male gender (%)	59.1	63.8	63.4
Current smokers (%)	9.1	7.8	7.7
BMI (kg/m^{-2})	29.9±5.2	29.1±54.7	28.7±4.9
Metabolic factors			
HbA1c (mmol/mmol)	49.9 (39.0-60.2)	48.0 (40.0-58.1)	47.0 (41.0-57.8)
LDL (mmol/L)	2.28±0.97	2.44±0.94	2.45±0.87
HDL (mmol/L)	1.24±0.44**	1.38±0.42	1.28±0.40
TG (mmol/L)	1.50 (1.00-2.10)	1.30 (0.93-1.85)	1.30 (0.90-1.86)
Blood pressure			
Systolic (mmHg)	137±18	134±17	137±16
Diastolic (mmHg)	77±9*	75±9	77±10
Renal function			
eGFR	77.9±20.7	80.1±19.0	82.2±19.3

Variables with normal distribution are shown as mean±standard deviation and skewed variables as median and interquartile range. eGFR (mL/min^{-1} per $1.73m^2$). Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and significance of differences versus no change with Scheffe's post hoc test. Chi-square test was used to analyze difference between categorical variables. *p<0.05, **p<0.01 and ***p<0.001.

Supplemental table 4. Relationships between biomarkers and change in carotid bulb IMT using ±0.1 mm as cut-off for no change in IMT. Related to table 3.

	Regression (n=137)	No change (n=367)	Progression (n=302)
Inflammatory			
hsCRP (mg/L)	1.48 (0.69-3.15)	1.12 (0.60-2.32)	1.46 (0.75-2.90)
IL-6	42.5 (31.3-66.3)**	31.8 (22.2-48.2)	34.5 (23.7-56.2)
MCP-1	14.2 (10.9-18.0)***	11.4 (8.6-14.7)	11.5 (9.0-14.9)
MIP-1α	5.13 (4.22-6.50)**	4.71 (3.97-5.39)	4.59 (3.86-5.66)
Matrix proteases			
MMP-3	2.51 (1.91-3.40)	2.38 (1.90-2.97)	2.38 (1.91-3.03)
MMP-7	468 (282-676)	387 (283-559)	446 (311-690)*
MMP-12	154 (99-232)**	122 (91-169)	136 (98-199)*
Apoptosis			
TNFR-1	6608 (5185-8192)*	6039 (5008-7473)	6295 (5185-7643)
TRAILR-2	3.68 (2.51-4.63)*	3.24 (2.35-4.21)	3.18 (2.31-4.33)
Fas	226 (177-280)*	197 (168-242)	198 (167-244)
SMC Growth factors			
PDGF	70.0 (29.2-166.8)***	132.5 (61.2-246.0)	174.8 (90.4-263.2)*
EGF	35.6 (11.0-73.5)***	51.1 (21.1-116.2)	67.6 (32.4-121.1)*
HBEGF	22.8 (18.9-29.0)**	25.9 (21.0-34.8)	26.7 (21.6-34.5)
EC growth factors			
HGF	121 (93-150)**	101 (83-130)	103 (87-127)
PlGF	191 (144-246)**	163 (139-202)	168 (139-207)
VEGF	1510 (1193-1965)	1370 (1097-1783)	1370 (1097-1739)

All biomarkers except hsCRP are expressed as arbitrary units and values are shown as median and IQR. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. If correcting for multiple comparisons in the present table, the threshold for significance is $p<0.004$. SMC; smooth muscle cell, EC; endothelial cell.

Supplemental table 5. Relationships between biomarkers and change in common carotid IMT using ±0.1 mm as cut-off for no change in IMT. Related to table 4.

	Regression (n=181)	No change (n=379)	Progression (n=299)
Inflammatory			
hsCRP (mg/L)	1.12 (0.62-1.90)	1.33 (0.66-2.87)	1.20 (0.70-2.53)
IL-6	40.8 (24.1-61.0)	34.9 (23.2-53.1)	36.9 (25.4-61.0)
MCP-1	13.4 (10.5-16.1)	11.5 (8.8-15.5)	12.1 (9.7-15.6)
MIP-1α	4.77 (3.79-6.00)	4.72 (3.95-5.74)	4.94 (4.00-5.99)
Matrix proteases			
MMP-3	2.60 (1.95-3.13)	2.36 (1.89-3.07)	2.62 (1.92-3.34)
MMP-7	440 (220-709)	422 (296-633)	471 (335-704)
MMP-12	143 (107-205)	133 (97-201)	144 (100-207)
Apoptosis			
TNFR-1	6427 (5293-8079)	6339 (5149-7804)	6295 (5104-7968)
TRAILR-2	3.89 (3.05-4.87)**	3.32 (2.37-4.32)	3.11 (2.42-4.19)
Fas	219 (173-267)	204 (169-247)	211 (170-272)
SMC Growth factors			
PDGF	84.4 (43.9-200.2)	144.0 (61.0-243.9)	133.9 (62.6-233.5)
EGF	41.1 (17.1-92.6)	53.8 (20.6-111.4)	51.2 (23.5-104.1)
HBEGF	24.1 (18.4-33.2)	25.3 (20.7-32.7)	26.4 (21.6-33.8)
EC growth factors			
HGF	110 (88-133)	106 (87-133)	114 (87-137)
PlGF	183 (144-218)	171 (141-214)	173 (144-228)
VEGF	1443 (1193-1786)	1339 (1121-1795)	1448 (1105-1761)

All biomarkers except hsCRP are expressed as arbitrary units and values are shown as median and IQR. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. If correcting for multiple comparisons in the present table, the threshold for significance is $p<0.004$. SMC; smooth muscle cell, EC; endothelial cell.

Supplemental table 6. Relationships between baseline vascular measurements and change in carotid IMT using ±0.1 mm as cut-off for no change in IMT. Related to table 5.

	Carotid bulb		
	Regression	No change	Progression
CCA	n=191	n=200	n=412
Mean IMT (mm)	0.88 (0.81-1.05)	0.86 (0.75-0.99)	0.89 (0.78-1.03)
Bulb			
Mean IMT (mm)	1.31 (1.10-1.69)***	1.02 (0.87-1.19)	1.10 (0.90-1.40)
GSM baseline	40 (28-63)***	66 (48-83)	69 (50-90)
GSM follow-up	50 (35-70)***	67 (49-92)	73 (50-96)
Arterial stiffness			
PWV (m/s)	10.9 (9.3-13.4)**	10.0 (8.6-11.9)	10.5 (8.9-12.5)
Endothelial function			
RHI	2.03 (1.75-2.47)*	2.19 (1.79-2.66)	2.13 (1.79-2.62)
	CCA		
CCA	n=181	n=379	n=299
Mean IMT (mm)	1.03 (0.88-1.24)***	0.87 (0.77-0.99)	0.85 (0.74-0.99)
Bulb			
Mean IMT (mm)	1.10 (0.94-1.51)	1.06 (0.91-1.31)	1.10 (0.93-1.39)
GSM baseline	58 (36-79)	64 (43-83)	67 (46-88)
GSM follow-up	54 (43-71)	66 (46-87)	62 (47-87)
Arterial stiffness			
PWV (m/s)	10.8 (9.6-13.6)	10.2 (8.7-12.4)	10.6 (9.0-12.6)
Endothelial function			
RHI	1.98 (1.74-2.39)	2.14 (1.76-2.64)	2.16 (1.88-2.69)

Grey scale median (GSM) value is the median of all grey levels of the pixels in the plaque. PWV; pulse wave velocity, RHI; reactive hyperemia index. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. Values are shown as median and IQR. *p<0.05, **p<0.01 and ***p<0.001.

Supplemental table 7. Relationships between biomarkers and change in carotid bulb IMT in subjects with T2D. Related to table 3.

	Correlation	P	Regression (n=126)	No change (n=125)	Progression (n=269)
Inflammatory					
hsCRP (mg/L)	0.06	ns	1.42 (0.66-3.30)	1.65 (0.65-2.96)	1.56 (0.73-3.00)
IL-6	0.01	ns	41.4 (29.4-68.1)	33.4 (24.9-52.7)	36.3 (26.0-57.3)
MCP-1	-0.05	ns	13.7 (9.8-17.3)	11.4 (8.6-15.3)	12.0 (9.4-15.6)
MIP-1α	-0.07	ns	4.89 (3.97-6.11)	4.68 (3.92-5.36)	4.66 (3.94-5.74)
Matrix proteases					
MMP-3	0.00	ns	2.51 (1.91-3.40)	2.38 (1.90-2.97)	2.38 (1.91-3.03)
MMP-7	0.12	0.005	452 (233-779)	405(304-564)	495 (341-719)*
MMP-12	0.05	ns	154 (100-223)	128 (95-183)	151 (108-211)
Apoptosis					
TNFR-1	0.04	ns	6517 (5185-8422)	6320 (5113-7486)	6700 (5367-8192)
TRAILR-2	-0.08	ns	3.88 (2.59-5.07)	3.36 (2.57-4.32)	3.36 (2.48-4.53)
Fas	-0.08	ns	231 (176-286)	205 (168-247)	208 (175-251)
SMC Growth factors					
PDGF	0.28	8.8E-11	75.1 (33.3-164.3)**	137.7 (65.3-231.5)	177.3 (90.4-263.7)*
EGF	0.24	3.9E-8	33.8 (12.6-74.5)*	54.0 (25.1-106.0)	68.6 (32.4-123.6)
HBEGF	0.13	0.003	22.5 (18.5-29.2)	26.1 (20.7-33.6)	26.9 (21.6-34.3)
EC growth factors					
HGF	-0.04	ns	123 (92-153)	104 (87-130)	110 (92-134)
PlGF	-0.01	ns	187 (143-246)	161 (142-198)	174 (146-217)
VEGF	-0.03	ns	1531 (1184-2091)	1389 (1142-1827)	1448 (1144-1808)

All biomarkers except hsCRP are expressed as arbitrary units and values are shown as median and IQR. Correlations are shown as Spearman rank correlation coefficients. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. If correcting for multiple comparisons in the present table, the threshold for significance is $p<0.004$. SMC; smooth muscle cell, EC; endothelial cell.

Supplemental table 8. Relationships between biomarkers and change in carotid bulb IMT in subjects without T2D. Related to table 3.

	Correlation	P	Regression (n=65)	No change (n=75)	Progression (n=143)
Inflammatory					
hsCRP (mg/L)	-0.04	ns	1.38 (0.70-2.55)	0.84 (0.60-2.06)	0.93 (0.60-2.01)
IL-6	-0.10	ns	35.3 (25.1-54.4)	26.7 (18.6-44.1)	25.5 (18.3-46.7)
MCP-1	-0.19	0.002	13.0 (10.1-16.1)	11.7 (8.5-16.3)	10.5 (8.4-13.2)
MIP-1α	-0.22	3.4E-4	5.24 (4.32-6.64)*	4.77 (3.97-5.48)	4.42 (3.74-5.39)
Matrix proteases					
MMP-3	-0.13	0.03	2.69 (1.90-3.42)	2.41 (2.03-3.07)	2.23 (1.77-2.87)
MMP-7	-0.08	ns	503 (322-628)	353(272-530)	384 (293-547)
MMP-12	-0.12	0.04	153 (96-238)**	116 (84-145)	117 (85-178)
Apoptosis					
TNFR-1	-0.15	0.01	6700 (5230-7954)*	5673 (4656-7145)	5576 (4713-6865)
TRAILR-2	-0.17	0.004	3.52 (2.36-4.37)	2.87 (2.03-4.00)	2.76 (1.99-3.51)
Fas	-0.22	2.6E-4	224 (181-271)	191 (166-241)	184 (159-222)
SMC Growth factors					
PDGF	0.27	6.0E-6	67.6 (26.9-177.3)**	124.5 (51.3-286.5)	168.3 (89.6-257.8)
EGF	0.25	2.5E-5	31.8 (7.09-72.8)**	46.9 (17.6-128.7)	65.1 (31.1-113.4)
HBEGF	0.15	0.01	24.0 (20.6-28.3)*	25.2 (21.5-39.9)	26.4 (22.0-35.2)
EC growth factors					
HGF	-0.23	1.3E-4	113 (93-138)*	97 (72-125)	92 (75-109)
PlGF	-0.22	2.9E-4	192 (167-248)*	167 (135-231)	155 (134-187)
VEGF	-0.16	0.007	1468 (1220-1898)	1342 (1029-1761)	1269 (1038-1593)

All biomarkers except hsCRP are expressed as arbitrary units and values are shown as median and IQR. Correlations are shown as Spearman rank correlation coefficients. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. If correcting for multiple comparisons in the present table, the threshold for significance is $p<0.004$. SMC; smooth muscle cell, EC; endothelial cell.

Supplemental table 9. Relationships between biomarkers and change in CCA IMT in subjects with T2D. Related to table 4.

	Correlation	P	Regression (n=122)	No change (n=256)	Progression (n=186)
Inflammatory					
hsCRP (mg/L)	0.05	ns	1.40 (0.69-3.60)	1.45 (0.66-3.10)	1.67 (0.86-3.00)
IL-6	0.01	ns	41.3 (26.0-64.7)	36.8 (27.3-55.7)	38.6 (26.3-59.3)
MCP-1	-0.08	ns	13.4 (10.2-16.8)	11.5 (9.1-15.9)	12.2 (9.4-15.7)
MIP-1α	0.01	ns	4.86 (3.77-5.83)	4.72 (4.00-5.66)	4.72 (3.97-5.84)
Matrix proteases					
MMP-3	-0.04	ns	2.68 (2.04-3.32)	2.31 (1.91-3.05)	2.51 (1.94-3.16)
MMP-7	0.12	0.004	399 (193-696)**	449 (308-686)	495 (347-718)
MMP-12	0.01	ns	1347(101-209)	149 (105-207)	147 (103-215)
Apoptosis					
TNFR-1	-0.06	ns	6608 (5557-8452)	6700 (5176-8135)	6562 (5221-8023)
TRAILR-2	-0.18	<0.0001	4.04 (3.11-5.06)**	3.43 (2.46-4.53)	3.27 (2.48-4.35)
Fas	-0.02	ns	215 (175-267)	208 (175-244)	212 (171-266)
Growth factors					
PDGF	0.09	0.037	88.0 (41.1-215.3)*	144.0 (67.2-247.7)	153.3 (72.0-245.6)
EGF	0.02	ns	42.5 (16.7-111.3)	55.3 (24.4-111.4)	58.1 (21.5-108.8)
HBEGF	0.06	ns	24.1 (18.5-33.4)	25.3 (20.5-32.7)	26.2 (20.7-33.6)
EC growth factors					
HGF	-0.04	ns	117 (93-143)	111 (92-136)	111 (91-141)
PlGF	-0.01	ns	184 (145-243)	171 (144-212)	167 (144-221)
VEGF	-0.03	ns	1520 (1209-1868)	1489 (1168-1898)	1389 (1128-1758)

All biomarkers except hsCRP are expressed as arbitrary units and values are shown as median and IQR. Correlations are shown as Spearman rank correlation coefficients. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. *p<0.05, **p<0.01 and ***p<0.001. If correcting for multiple comparisons in the present table, the threshold for significance is p<0.004. SMC; smooth muscle cell, EC; endothelial cell.

Supplemental table 10. Relationships between biomarkers and change in CCA IMT in subjects without T2D. Related to table 4.

	Correlation	P	Regression (n=59)	No change (n=122)	Progression (n=113)
Inflammatory					
hsCRP (mg/L)	-0.04	ns	0.96 (0.63-1.69)	0.98 (0.60-2.20)	0.95 (0.60-2.12)
IL-6	-0.04	ns	35.3 (21.6-50.6)	29.2 (18.3-46.4)	31.3 (20.0-47.8)
MCP-1	0.02	ns	11.8 (8.9-14.8)	11.2 (8.5-14.3)	11.6 (8.7-14.8)
MIP-1α	-0.01	ns	4.79 (3.93-5.80)	4.77 (4.02-6.02)	4.69 (3.92-5.82)
Matrix proteases					
MMP-3	0.06	ns	2.35 (1.93-2.99)	2.40 (1.79-3.04)	2.34 (1.90-3.35)
MMP-7	-0.12	0.05	501 (301-664)	392 (292-614)	383 (276-541)
MMP-12	-0.04	ns	1347(101-209)	149 (105-207)	147 (103-215)
Apoptosis					
TNFR-1	-0.06	ns	6383 (4873-7697)	5894 (4999-7345)	5873 (4837-7082)
TRAILR-2	-0.07	ns	3.48 (2.41-4.18)	2.85 (1.97-3.86)	2.85 (2.32-3.72)
Fas	-0.02	ns	202 (159-236)	195 (162-238)	194 (164-239)
Growth factors					
PDGF	0.05	ns	122.8 (43.0-200.9)	145.0 (58.3-254.2)	115.4 (57.3-240.5)
EGF	-0.03	ns	50.2 (16.6-108.8)	44.9 (20.6-115.2)	47.5 (18.9-94.4)
HBEGF	0.01	ns	25.6 (21.5-33.4)	25.6 (21.4-32.8)	24.9 (21.9-34.5)
EC growth factors					
HGF	-0.01	ns	101 (82-123)	96 (78-122)	96 (78-123)
PlGF	-0.04	ns	177 (146-226)	174 (140-208)	170 (134-218)
VEGF	0.01	ns	1389 (1105-1652)	1342 (1044-1675)	1389 (1053-1687)

All biomarkers except hsCRP are expressed as arbitrary units and values are shown as median and IQR. Correlations are shown as Spearman rank correlation coefficients. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. If correcting for multiple comparisons in the present table, the threshold for significance is $p<0.004$. SMC; smooth muscle cell, EC; endothelial cell.

Supplemental table 11. Relationships between baseline vascular measurements and change in carotid bulb IMT. Related to table 5.

	T2D				
	Correlation	P	Regression	No change	Progression
CCA			n=126	n=125	n=269
Mean IMT (mm)	0.03	ns	0.89 (0.80-1.02)	0.88 (0.77-0.98)	0.86 (0.74-0.99)
Bulb					
Mean IMT (mm)	0.02	ns	1.23 (1.01-1.63)***	1.06 (0.89-1.23)	1.11 (0.94-1.37)
GSM baseline	0.16	0.001	43 (31-69)***	65 (49-84)	67 (46-85)
GSM follow-up	0.23	6.9E-5	51 (35-68)**	67 (49-91)	60 (46-83)
Arterial stiffness					
PWV (m/s)	0.12	ns	11.2 (9.3-13.3)	10.2 (8.9-12.3)	10.7 (9.2-12.6)
Endothelial function					
RHI	0.00	ns	1.97 (1.68-2.45)	2.11 (1.66-2.58)	2.04 (1.65-2.57)
	Non-T2D				
CCA			n=65	n=75	n=143
Mean IMT (mm)	0.00	ns	0.86 (0.80-1.02)	0.83 (0.74-0.984	0.87 (0.75-0.98)
Bulb					
Mean IMT (mm)	-0.16	0.01	1.21 (1.01-1.49)***	0.99 (0.87-1.16)	1.01 (0.87-1.26)
GSM baseline	0.25	1.9E-4	45 (31-78)**	68 (49-94)	69 (56-87)
GSM follow-up	0.23	0.007	59 (43-75)	70 (55-87)	72 (50-108)
Arterial stiffness					
PWV (m/s)	-0.07	ns	10.2 (9.2-12.6)**	9.0 (7.9-10.4)	9.7 (8.3-11.4)
Endothelial function					
RHI	-0.03	ns	2.31 (1.91-2.83)	2.36 (2.04-2.71)	2.38 (1.90-2.75)

Grey scale median (GSM) value is the median of all grey levels of the pixels in the plaque. PWV; pulse wave velocity, RHI; reactive hyperemia index. Correlations are shown as Spearman rank correlation coefficients. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. Values are shown as median and IQR. *p<0.05, **p<0.01 and ***p<0.001.

Supplemental table 12. Relationships between baseline vascular measurements and change in CCA IMT. Related to table 5.

	T2D				
	Correlation	P	Regression (n=122)	No change (n=256)	Progression (n=186)
CCA					
Mean IMT (mm)	-0.23	4.2E-8	0.98 (0.86-1.15)***	0.88 (0.77-0.99)	0.86 (0.74-1.00)
Bulb					
Mean IMT (mm)	-0.02	ns	1.13 (0.94-1.45)	1.06 (0.93-1.34)	1.11 (0.94-1.37)
GSM baseline	0.13	0.007	51 (37-79)	62 (44-84)	67 (46-85)
GSM follow-up	0.12	0.034	53 (42-73)	68 (43-91)	60 (46-83)
Arterial stiffness					
PWV (m/s)	-0.09	ns	11.6 (9.9-13.7)	10.5 (8.9-13.1)	10.7 (9.2-12.6)
Endothelial function					
RHI	0.05	ns	1.97 (1.70-2.43)	2.03 (1.67-2.49)	2.06 (1.75-2.47)
	Non-T2D				
			Regression (n=126)	No change (=125)	Progression (n=269)
CCA					
Mean IMT (mm)	-0.24	3.1E-5	0.94 (0.83-1.12)***	0.85 (0.75-0.98)	0.83 (0.73-0.92)
Bulb					
Mean IMT (mm)	0.03	ns	1.05 (0.90-1.35)	1.03 (0.88-1.29)	1.06 (0.87-1.30)
GSM baseline	-0.03	0.001	63 (37-78)	70 (46-8)	60 (40-82)
GSM follow-up	0.03	6.9E-5	62 (48-75)	70 (48-92)	64 (49-86)
Arterial stiffness					
PWV (m/s)	-0.12	0.04	10.2 (9.2-11.5)	9.5 (8.3-11.6)	9.5 (8.2-11.0)
Endothelial function					
RHI	0.00	ns	2.26 (1.83-2.74)	2.44 (1.99-2.80)	2.35 (1.99-2.74)

Grey scale median (GSM) value is the median of all grey levels of the pixels in the plaque. PWV; pulse wave velocity, RHI; reactive hyperemia index. Correlations are shown as Spearman rank correlation coefficients. Differences between IMT change categories (regression and progression versus no change) were calculated with one-way ANOVA and Scheffe's post hoc test. Values are shown as median and IQR. *p<0.05, **p<0.01 and ***p<0.001.

Supplemental table 13. Biomarkers in T2D subjects without and with insulin treatment. Related to table 3.

	No insulin (n=419)	Insulin (n=143)	P
Inflammatory			
hsCRP (mg/L)	1.43 (0.67-2.94)	1.80 (0.84-3.51)	0.04
IL-6	36.6 (25.4-56.2)	41.6 (31.8-64.9)	0.007
MCP-1	11.9 (9.2-15.9)	12.6 (10.0-16.3)	ns
MIP-1α	4.68 (3.95-5.67)	4.92 (4.00-5.90)	ns
Matrix proteases			
MMP-3	2.45 (1.97-3.05)	2.27 (1.87-3.27)	ns
MMP-7	428 (290-665)	532(356-733)	0.001
MMP-12	140 (101-205)	170 (115-232)	0.003
Apoptosis			
TNFR-1	6427 (5221-7968)	7231 (5595-8964)	0.001
TRAILR-2	3.40 (2.52-4.53)	3.76 (2.71-5.31)	0.02
Fas	211 (175-256)	208 (168-263)	ns
SMC Growth factors			
PDGF	137.2 (57.3-242.2)	160.9 (71.0-240.5)	ns
EGF	51.1 (19.8-104.0)	61.0 (29.4-124.5)	0.045
HBEGF	25.5 (19.8-33.1)	25.3 (20.0-32.7)	ns
EC growth factors			
HGF	110 (92-137)	120 (83-144)	ns
PlGF	171 (143-214)	177 (146-228)	ns
VEGF	1428 (1160-1833)	1563 (1209-1859)	ns

All biomarkers except hsCRP are expressed as arbitrary units and values are shown as median and IQR. Comparisons between the groups were done using the Mann-Whitney U test. If correcting for multiple comparisons in the present table, the threshold for significance is $p<0.004$. SMC; smooth muscle cell, EC; endothelial cell.

Supplemental table 14. Vascular measurements in T2D subjects without and with insulin treatment. Related to table 5.

	No insulin (n=419)	Insulin (n=143)	P
Bulb baseline IMT (mm)	1.06 (0.93-1.31)	1.22 (1.00-1.56)	<0.001
CCA baseline IMT (mm)	0.89 (0.77-1.03)	0.90 (0.81-1.03)	ns
Progression bulb IMT (mm)	0.06 (-0.03-0.17)	0.06 (-0.11-0.20)	ns
Progression CCA IMT (mm)	0.02 (-0.04-0.08)	0.00 (-0.05-0.06)	(0.05)
GSM baseline	64 (44-89)	59 (39-79)	ns
GSM follow-up	61 (44-84)	62 (43-85)	ns
PWV (m/s)	10.5 (8.9-12.8)	12.0 (9.9-14.0)	<0.001
RHI	2.05 (1.73-2.51)	1.97 (1.65-2.35)	ns

Grey scale median (GSM) value is the median of all grey levels of the pixels in the plaque. PWV; pulse wave velocity, RHI; reactive hyperemia index. Comparisons between the groups were done using the Mann-Whitney U test.