Vascular structural and functional changes associated with clinically manifest cardiovascular disease in type 2 diabetes

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Abstract

Background – There is a need to develop and validated surrogate markers of cardiovascular disease (CVD) in diabetes. The macrovascular changes associated with diabetes include an aggravated atherosclerosis, increased arterial stiffness and endothelial dysfunction. In the present study we aimed to determine which of these factors are most effective at identifying patients who have a clinically manifest cardiovascular event.

Methods and results – We measured carotid intima media thickness (IMT), ankle-brachial pressure index (ABPI), pulse wave velocity (PWV) and endothelial function assessed by the reactive hyperemia index (RHI) in a cohort of 458 subjects with type 2 diabetes (T2D) and CVD (myocardial infarction, stroke or lower extremity arterial disease), 527 subjects with T2D but no clinically manifest CVD and 515 non-T2D subjects with or without CVD. Carotid IMT and ABPI demonstrated independent association with the presence of clinical CVD in T2D, while assessment of PWV and RHI provide limited independent additive information. Measurement of IMT in the bulb provided a better discrimination of the presence of CVD in T2D than measurement of IMT in the common carotid artery. The most important factors associated with increased carotid IMT in T2D were age, diabetes duration, systolic blood pressure, impaired renal function and increased arterial stiffness, whereas no or only weak independent associations were found with metabolic factors and endothelial dysfunction.

Conclusions - Our findings demonstrate that measures of atherosclerotic burden show the best association with clinically manifest CVD in T2D. They also show that vascular changes not directly related to known metabolic risk factors are of importance in atherosclerosis and CVD in T2D. A better understanding of these mechanisms will be of critical importance for development of more effective preventive cardiovascular therapies in diabetes.

Cardiovascular disease (CVD) is a major cause of morbidity and mortality among subjects with diabetes.[1-3] The risk of myocardial infarction (MI) is increased 2-3 fold in subjects with duration of diabetes longer than 10 years with some studies reporting risks equivalent to that of a non-diabetic person with a previous myocardial infarction.[2] Diabetes is also associated with an increased risk of stroke and lower extremity arterial disease (LEAD), increased risk of complications after acute CVD events as well as with an increased risk for recurrent CVD events.[1] Prevention of cardiovascular (CV) complications in diabetes represents an increasingly important medical and socioeconomic challenge. Intensive glycemic control has been found to provide protection against development of microvascular complications in diabetes and macrovascular disease in type 1 diabetes but the effect on macrovascular complications in type 2 diabetes is less clear.[4-6] New generations of anti-diabetic therapies including, glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors are currently being evaluated as to whether they reduce CVD in type 2 diabetes (T2D), but have so far failed to demonstrate significant protective effects.[7-9] Observations from long-term follow-up of the Diabetes Control and Complication Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) suggest a lowering of CV risk that becomes evident first after several years of intense glycemic control.[10, 11] These findings suggest that CV complications in diabetes involve long-term functional and structural changes in the vasculature rather than more short-term metabolic and/or pro-inflammatory effects. They also point to the need for gaining a better understanding of CVD mechanisms in diabetes.

The Innovative Medicine Initiative project SUMMIT (SUrrogate markers for Micro- and Macro-vascular hard endpoints for Innovative diabetes Tools) was initiated to develop and validate genetic markers, circulating biomarkers and imaging techniques that can identify risk for cardiovascular complications in diabetes and that can be used to monitor response to therapy. The primary aim of the present SUMMIT study was to identify vascular changes associated with clinically manifest CVD in T2D. Non-diabetic subjects with or without CVD were also included in the study to allow identification of diabetes-specific changes and to make it possible to determine if risk factors for vascular changes differ between subjects with and without T2D. The study population consisted of 458 subjects with T2D and CVD, 527 subjects withT2D but no clinically manifest CVD and 515 non-T2D subjects (245 with CVD and 270 without CVD) recruited at four different European centers. The left and right intima-media media thickness (IMT) of the common carotid arties (CCA) and the carotid bulbs were used as surrogate markers of carotid atherosclerosis and the ankle-brachial pressure index

(ABPI) as a surrogate marker of atherosclerosis in the lower extremities. Pulse wave velocity (PWV) was used to determine arterial stiffness and endothelial function assessed by the reactive hyperemia index using the Endo-Peripheral Arterial Tone (EndoPAT) technology. Our observations show that vascular changes associated with CVD inT2D are best assessed by measuring ABPI and IMT in the right carotid artery.

Methods

Study population

The study cohort included 458 subjects with T2D and clinically manifests CVD, 527 with T2D but without clinical signs of CVD, 245 with CVD but no diabetes and 270 without both CVD and T2D recruited from existing population cohorts and hospital registers at the university hospitals in Malmö, Pisa, Dundee and Exeter. Subjects with a diagnosis of T2D before the age of 35 years or insulin treatment during the first 12 months after diagnosis of T2D were not included the study. Classification of CVD included non-fatal acute myocardial infarction, hospitalized unstable angina, resuscitated cardiac arrest, any coronary revascularization procedure, non-fatal stroke, transient ischemic attack (TIA) confirmed by specialist, lower extremity arterial disease (LEAD) defined as ABPI <0.9 with intermittent claudication or prior corrective surgery, angioplasty or above ankle amputation. T2D with and without CVD were matched for gender, age (± 5 years) and duration of diabetes (± 5 years). Subjects without T2D were matched for gender and age (± 5 years). CVD subjects with or without T2D were matched for CVD complication type. Subjects with renal replacement therapy, malignancy requiring active treatment, end-stage renal disease, any chronic inflammatory disease on therapy, previous bilateral carotid artery invasive interventions or age < 40 years were not included in the study. The study was approved by the local ethical review boards and all study subjects provided written informed consent.

Ultrasound

An ultrasound examination of the carotid arteries was performed for assessment of atherosclerotic status. The subject was in a supine position with the head turned approximately 90 degrees away from the examined side. End-diastolic images of the artery,

captured on the top of the R wave of an ECG (lead I) simultaneously shown on the screen, were saved for off-line measurement of IMT. IMT was measured both in CCA and in the bulb, the beginning of the bulb set to be where the far wall began to form a curvature. Images were taken striving to get the echoes representing the transitions between lumen and intima and media and adventitia in the far wall sharp over at least one centimetre of the CCA and bulb respectively. In CCA also the echoes representing the intima-lumen transition was to be sharp, to ensure that images were taken perpendicular to the artery. All images were taken in the projection showing the thickest IMT in the far wall at each site. IMT was measured in the far wall of the artery according to the leading edge principle, using a semiautomatic analysis system, Artery Measurement Software (AMS).[12] The thickness of the intima-media complex was measured as the distance between the leading edges of the echoes representing lumen-intima and media-adventitia transitions. The echoes were automatically outlined in the program, with the possibility for the observer to make manual adjustments when needed. The computer measured the distance between the lines at approximately 100 sites over each one centimetre section and values for mean, median, maximum and minimum IMT were automatically calculated. IMT values for CCA and bulb are presented as the mean thickness of the intima-media complex in the far wall, just proximal and distal, respectively, to the beginning of the bulb. Before the start of the study sonographers from the four centres participated in an assessment of inter-observer variability. Images were taken for measurement of IMT in CCA and in the bulb using the same machine. A total of 17 subjects were examined by a sonographer from each centre. The examinations were performed without any knowledge of the result from the other sonographers. The absolute difference between centres for IMT in CCA was 0.089 mm (10.1 %), range 0.07 – 0.11 mm and for IMT bulb it was 0.17 mm (14.1 %), range 0.10 – 0.24 mm. Further training to decrease inter-observer variability was performed throughout the study.

Measurements of endothelial function and arterial stiffness

The subjects were asked to refrain from coffee and tea for at least 2 hours, nicotine for at least 4 hours and alcohol intake for at least 12 hours before the investigation. Only a light meal was allowed during the previous 3 hours. The examination was performed in a quiet room, with a temperature between 21 and 24°C. The subjects were in a supine position with restrictive clothing as well as watches and jewellery on the hands removed.

Endothelial function was measured using an EndoPat (Itamar Medical, Caesarea Ind. Park, Israel), estimating the endothelium-dependent vasodilation following post-ischemic hyperaemia. A cuff was placed on the non-dominant upper arm. The index-fingers or middle fingers were placed in pneumo-electric tubes. Arterial pulsatile volume changes from both hands were recorded continuously. After 10 minutes of rest the cuff was inflated to 200 mmHg, with the opportunity to increase the pressure to a maximum of 300 mmHg if necessary. After five minutes of occlusion the pressure of the cuff was released and the arterial dilation mediated by the occlusion, assessed as an increase in the signal amplitude, were recorded for another eight minutes. Reactive hyperaemia index (RHI) was calculated as a post-occlusion to pre-occlusion ratio of the signal amplitudes. Thirty-one subjects were excluded from the RHI analysis due to "Incomplete occlusion" or "NonStandOccLen". "Incomplete occlusion" was recorded if brachial pulses from the occluded arm were visible during occlusion, despite an increase of the pressure of the cuff to the maximum level of 300 mmHg. If the time of occlusion either exceeded or was less than five minutes "NonStandOccLen" was stated in the result file.

Arterial stiffness was assessed by calculating Pulse Wave Velocity (PWV) and Pulse Wave Analysis, using a Sphygmocor device (Atcor Medical, Australia). A blood pressure cuff was attached to the left arm, and three electrocardiographic electrodes (lead I) was attached. The carotid and femoral pulses were carefully located. The distance from the carotid pulse to fossa jugularis was measured and entered as proximal distance. The distal distance was entered as the combined distances of fossa jugularis to umbilicum and umbilicum to femoral pulse. The distance used for the calculation of PWV was distal minus proximal distance. After five minutes of rest the blood pressure was measured three times, with one minute between measurements. Mean value of the two final measurements was entered. The carotid and femoral pulses were captured with focus of getting a clear foot of the curves. PWV (m/s) was automatically calculated as the differences in time between the R wave of the ECG to the foot of the carotid and femoral pulse curves respectively, divided with the calculated distance. Three measurements with good quality were saved.The radial pulse was captured for assessment of PWA (%). Observer index had ideally to be > 90%, but in case this was not possible > 80% was accepted. The blood pressure values from the PWV measurement were entered. When three PWA registrations of good enough quality had been saved, three new blood pressure measurements, one minute between, were done. Blood pressure values for the PWA registrations were subsequently changed to the mean of the two final values.

Ankle brachial index

Blood pressure cuffs in appropriate size were attached to the upper arms and the ankles. A sphygmomanometer was attached to the cuffs. The systolic blood pressure was measured using a 5-10 MHz Doppler probe. The blood pressure in the arms was measured over the brachial arteries. For the ankle the posterior tibial artery and the dorsal artery of the foot was used. Blood pressures were measured in a horseshoe shape, beginning in the right arm, and continuing with right foot, left foot and finally left arm. Ankle brachial index was calculated as the ratio between the highest systolic blood pressure values from each foot respectively and the blood pressure from the arm giving the highest value.

Statistics

Differences in characteristics between CVD and T2D groups were investigated using Chi2 or Kruskal–Wallis (Mann–Whitney U) tests, as appropriate. For continuous traits median and interquartile ranges are reported and correlations were estimated using Spearman's rho. Two different logistic regression models were used to test for associations between CVD and T2D groups. The first model was adjusted for age, gender, statin use, anti-hypertensive treatment and current smoking. The second model was also adjusted for BMI, HDL, serum creatinine, HbA1c and SBP (SBP not included in blood pressure analyses and serum creatinine not included in eGFR models). The comparisons between T2D and Non-T2D were also adjusted for CVD status and the comparisons between CVD and Non-CVD in T2D for diabetes duration. Differences in CVD risk effect estimates between T2D and nonT2D groups were evaluated by incorporating an interaction terms between T2D status and phenotype. Interaction effects were evaluated in the second logistic regression model, excluding HbA1c and serum creatinine. The logistic regressions were performed stratified by center, followed by fixed-effect meta-analyses. Backward linear regression models were used to identify predictors that remain associated with vascular imaging variables in a multivariate setting. In these models dependent variables with a skewed distribution were logarithmically transformed and recruitment centre were included as covariates. Reported p-values are nominal. Because 11 imaging variables were tested across 4 different groups we consider p-

values below 0.05/44 = 0.0011 to be significant in the logistic regressions. Analyses were done using R version 2.15.2.

Results

The median age of the study population was 67.5 years and 979 (65%) were males. The baseline clinical characteristics of the study cohort are listed in table 1.

Vascular changes associated with CVD in subjects with T2D

T2D patients with CVD had a lower ABPI, increased IMT in the carotid bulb and the CCA, increased PWV, a higher pulse pressure and a lower RHI (table 2) than T2D patients without CVD. They were also older and had lower diastolic blood pressure (BP) as well as lower total, LDL and HDL cholesterol levels. Impaired renal function as assessed by serum creatinine, albumin creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR) was more common in T2D subjects with CVD. Also statin and anti-hypertensive treatment were more common in the T2D CVD group and they were also more often males and had a longer duration of T2D (table 2). The HbA1c levels were only marginally higher in the T2D CVD group and there were no significant differences in BMI, systolic BP or plasma triglycerides between T2D subjects with and without CVD.

When controlling for age, gender, current smoking as well as statin and anti-hypertensive treatment in logistic regression models ABPI (both right and left), carotid bulb IMT (both right and left), right CCA IMT, eGFR and diastolic blood pressure remained significantly associated with clinically manifest CVD in T2D (table 3). Notably, the predictive value of determining IMT in the right carotid artery was generally better than in the left carotid artery (table 3). The associations between presence of CVD and ABPI, carotid bulb IMT, eGFR and diastolic blood pressure remained significant when also controlling for anthropometry and metabolic factors characteristic for T2D including HDL cholesterol, systolic blood pressure, BMI and HbA1c in a second regression model (table 3). T2D patients with CVD had a lower RHI when controlling for age, gender, smoking as well as statin and anti-hypertensive treatment, but this difference did not remain significant when also controlling HDL cholesterol, systolic blood pressure, creatinine, diabetes duration, BMI and HbA1c (table 3).

These observations demonstrate that CVD in T2D primarily is associated with more advanced atherosclerosis. Interestingly, we did not detect the corresponding associations with CVD in individuals without diabetes (supplemental table 1). To evaluate if effect estimates differ between individuals with and without T2D interaction terms were fitted to the logistic regression models. The T2D interaction terms were nominally different for ABPI (right p=0.018 and left p=0.038), PWV (p=0.037), carotid bulb IMT (right p=5.0e-3 and left p=8.6e-3), right CCA IMT (p=6.4e-3), eGFR (7.7e-7) and diastolic blood pressure (7.4e-4).

Vascular changes associated with T2D

Subjects with T2D had increased IMT in the carotid bulb and the CCA, increased PWV and a lower RHI than those without T2D (table 4). There was no significant difference in ABPI between subjects with and without T2D. Subjects with T2D were more often on statin and anti-hypertensive treatment, had higher BMI, systolic BP, HbA1c and triglycerides, whereas total, LDL and HDL cholesterol levels were lower. The ACR was higher in T2D subjects but the serum creatinine and eGFR were not increased. The difference between subjects with and without T2D in the left and right CCA, systolic blood pressure, PWV and RHI remained significant when controlling for age, gender, current smoking, statin and anti-hypertensive treatment in a logistic regression model (table 5). However, all these associations were attenuated and became non-significant when also controlling for BMI, HDL, serum creatinine, systolic blood pressure (not included in the blood pressure analysis) and HbA1c in a second set of regression models (table 5).

Associations between different types of vascular changes

Increased carotid bulb and CCA IMT correlated with a higher PWV and a lower ABPI in analysis including all study subjects (table 6). When analyzing subjects with T2D separately and controlling for all covariates, PWV remained significantly associated with carotid IMT ($p<0.0005$ for right CCA and bulb and $p<0.05$ for left CCA and bulb, respectively). Notably, although the associations between carotid IMT and ABPI were highly significant they were still relatively weak suggesting a lack of strong correlation between atherosclerosis in the carotid and lower extremity arteries. The RHI showed a weak inverse association with left carotid bulb IMT (table 6), otherwise the RHI did not demonstrate significant associations

with vascular measurements reflecting atherosclerosis burden. These associations were generally the same when subjects with and without T2D were analyzed separately (data not shown).

Associations between risk factors and vascular changes

In T2D subjects CCA and bulb IMT were found to correlate with age, systolic BP and impaired renal function (table 7). A correlation with duration of diabetes was observed for carotid bulb but not for CCA IMT. CCA and carotid bulb IMT remained independently associated with age and systolic BP both in backward linear regression while the association with impaired renal function was lost when adjusting for age, gender and other cardiovascular risk factors (supplemental table 2). There were no significant independent associations between HbA1c levels and carotid IMT in subjects with T2D. Except for a weak inverse association between total cholesterol and IMT in the right carotid bulb there were also no significant independent associations between plasma lipids and carotid IMT in subjects with T2D in unadjusted correlation analyses. However, some weak associations between lipoproteins and carotid IMT could be identified when adjusting for covariates in the regression model (supplemental table 2). LDL cholesterol levels have been shown to correlate with CCA IMT in several population studies[13-15]. To determine if the weak association in the present study could be explained by the fact that many T2D subjects were on statin therapy we analyzed statin users (n=722) and non-users (n=260) separately. However, no significant associations between LDL and carotid IMT or ABPI could be identified in either of the two groups (data not shown). Diastolic BP correlated inversely with IMT in the carotid bulb but showed no association with CCA IMT (table 7).

The ABPI demonstrated significant association with systolic BP and to some extent also with renal function. However, there were no significant association with age and duration of diabetes (table 7). ABPI was also lower in smokers than in non-smokers ($p<2e-8$ for both). For PWV the strongest correlations were found with age and systolic BP, but significant associations were also noted with BMI, diabetes duration, diastolic BP, HbA1c, HDL and renal function (table 7). High BMI and low HDL were associated with a low RHI, while a high systolic BP was associated with a high RHI. Most of these associations remained independently significant when analyzed in backward linear regression models (supplemental table 2). Correlations between risk factors and vascular changes were generally the same

among non-diabetic subjects as for those with T2D (table 8). However, unexpectedly associations of carotid IMT with HbA1c and HDL appear stronger in non-diabetic than in subjects with T2D.

Discussion

T2D is associated with several pathological changes in the vasculature including endothelial dysfunction, increased arterial stiffness and a more severe development of atherosclerosis. In the present study we made an integrated assessment of how each of these changes relate to the presence of clinically manifest CVD in T2D. We demonstrate that measurements of carotid IMT and ABPI show the strongest associations with clinically evident CVD in T2D, while assessment of PWV and RHI provide limited independent additive information. An additional observation of potential clinical importance is that measurement of IMT in the bulb provides a better discrimination of the presence of CVD in T2D than measurement of IMT in the CCA. The difference in IMT between T2D subjects with and without CVD was also greater in the bulb than in the CCA (1.0-1.4 versus 0.04 mm). Carotid IMT measurements are commonly performed in the CCA. However, the present observations imply that important clinical information may be missed by only studying CCA. Moreover, we also found that IMT measurements of the right carotid artery provide better discrimination of the presence of CVD in T2D than measurement of the left carotid. This phenomena was particularly prominent in the CCA where increased IMT on the right side associated with the presence of CVD with an odds ratio 2.59 (95% CI 1.28-5.24), while no significant associations were found for the left side. It should be noted that in the present study there was no significant difference in IMT between the right and the left carotid artery. The reason for the better discriminative value of right-sided IMT measurements remains to be fully understood, but could depend on both technical and pathophysiological factors. As far as we are aware there have been no previous systematic comparisons between left and right carotid IMT measurements.

As both an increased carotid IMT and a decreased ABPI are regarded as surrogate markers of atherosclerosis our findings support the notion that a more aggressive development of atherosclerosis is the main reason for the higher incidence of CVD in T2D. The most important determinants of increased carotid bulb IMT in T2D subjects were age, diabetes duration, high systolic BP and impaired renal function. Notably, anthropometric and metabolic factors characteristic for diabetes such as high BMI, HbA1c, triglycerides and low

HDL demonstrated stronger associations with carotid IMT in non-T2D than in T2D subjects. The associations between BP, metabolic factors and carotid IMT (primarily in the CCA) is well established from several large population studies.[13-16] However, these associations have previously not been extensively studied specifically in subjects with T2D. It could be reasonable to assume that since metabolic factors such as hypertriglyceridemia, low HDL and increased HbA1c are more common in T2D the associations with carotid IMT would be stronger but our findings show that this is not the case. The weak association between metabolic factors and carotid IMT observed in the present study suggest that other factors are more important for development of atherosclerosis in T2D. In line with this notion the difference in carotid IMT (with the exception of the left CCA) between T2D subjects with and without CVD remained significant when controlling for cardiovascular risk factors as well as for statin and anti-hypertensive treatment further supporting the involvement of additional factors in the atherosclerotic disease process in T2D. In line with these findings Kinouchi and coworkers recently reported a lack of correlation between metabolic factors and carotid IMT in a cohort of 167 subjects with diabetes.[17]

A decreased eGFR was found to be an independent predictor of CVD in T2D in this study. In the general population both an increased ACR and a decreased eGFR predict risk for cardiovascular death.[18] Despite the fact that diabetes is the leading cause of chronic kidney disease in the developed world and T2D subjects with impaired renal function have a greatly increased cardiovascular mortality recent meta-analysis have shown that the relative cardiovascular risk of impaired renal function is the same irrespective of the presence or absence of diabetes.[19] Accordingly, although nephropathy is a common complication in diabetes it does not appear to be a diabetes-specific risk factor for CVD. In line with several previous studies[20, 21] we also found a correlation between decreased eGFR and increased carotid IMT suggesting that impaired renal function may contribute to CVD by an aggravation of atherosclerosis.

The ability of CCA IMT to predict risk for CVD events in a general population is well documented with a 0.1 mm increase in IMT being associated with a hazard ratio of 1.15 (95% CI 1.12-1.17) for MI and 1.18 for stroke (95% CI 1.16-1.21) after adjusting for age, gender and other vascular risk factors in a meta-analysis of 8 large studies.[22] In a subanalysis on 4220 subjects with diabetes from an ongoing meta-analysis of 17 populations-based cohorts den Ruijter and coworkers found no additive predictive value of CCA IMT to Framingham risk

score.[23] The findings of the present study suggest that risk prediction by carotid IMT in diabetes may be improved by analyzing the bulb rather than the CCA.

Evidence from experimental studies has suggested that oxidative stress leading to endothelial dysfunction could be one important factor in diabetic vascular complications.[24] Endothelial dysfunction as assessed by a low RHI has also been associated with presence of coronary artery disease and risk for CVD events both in patients with and without diabetes.[25-28] However, in spite of clear evidence of presence of impaired endothelial function in T2D subjects as assessed by the RHI we found no correlation with surrogate markers of atherosclerosis such as carotid IMT and ABPI nor did the RHI discriminate between T2D subjects with and without CVD. T2D subjects with a low RHI were characterized by a high BMI and low HDL levels. Moreover, the difference in RHI between subjects with and without T2D was lost when adjusting for these factors suggesting that they have an important role in diabetic endothelial dysfunction. Although the present studies do not support a role for endothelial dysfunction, as assessed by the Endo-PAT RHI, in the progression of atherosclerosis and cardiovascular events in diabetes its involvement in earlier stages of the vascular disease processes cannot be excluded.

It is well established that diabetes is associated with increased arterial stiffness.[29] In accordance we found that subjects with T2D had significantly increased PWV. The increased arterial stiffness in T2D subjects correlated with BMI and HDL cholesterol levels and when adjusting for these factors the difference in PWV between subjects with or without diabetes was no longer significant suggesting that these factors are major determinants of the increased arterial stiffness in T2D. Increased PWV has been identified as a risk factor for future cardiovascular events in population-based studies.[30, 31] Although T2D subjects with CVD had higher PWV in the present study this difference did not remain significant when adjusting for age, gender, smoking, statin use and anti-hypertensive treatment. However, the PWV still remained significantly associated with carotid IMT when controlling for all other covariates suggesting that the processes mediating increased arterial stiffness and atherosclerosis are related in T2D.

There are some limitations of the present study that should be considered. Most importantly, the cross-sectional design of the study will not allow conclusions regarding association of vascular changes in T2D and risk for future development of CVD. Hence, it cannot be excluded that the vascular changes observed in the T2D CVD group has occurred subsequent

to the CV event and/or are influenced by changes in treatment after the event. The inverse association between LDL cholesterol and carotid IMT is most likely one example of such effects. Although we took care to standardize vascular measurements between the different centers participating in the study we cannot exclude the possibility that the results have been influenced by inter-center variability.

Conclusion

In this study we investigated the association between vascular changes and CVD in a large cohort of subjects with CVD. We demonstrate that T2D subjects that suffer from clinically manifest CVD are characterized by a more severe development of atherosclerosis as assessed by an increased carotid IMT and a lower ABPI. The most important factors associated with increased carotid IMT in T2D were age, diabetes duration, systolic blood pressure, impaired renal function and increased arterial stiffness whereas no or only weak associations were found with metabolic factors and endothelial dysfunction. These observations suggest that vascular changes not directly related to known metabolic risk factors are of importance for the development of atherosclerosis and CVD in T2D. A better understanding of these mechanisms will be of critical importance for development of more effective preventive cardiovascular therapies in diabetes.

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Table 1. Baseline clinical characteristics

Phenotype	Total	T2D with CVD	T2D without CVD	Non-T2D without CVD	Non-T2D with CVD	p-value
N	1500	458	527	270	245	---
Male sex, n(%)	979 (65)	331 (72)	324 (61)	163 (60)	161 (66)	0.0011
Age, years	67.5 (8.7)	69.3 (7.8)	66.2 (8.8)	66.4 (9.1)	67.9 (9.2)	1.6e-06
T2D Duration, years	10.1 (8.1)	12.2 (8.8)	9.1 (6.9)	-	-	3.4e-09
BMI, kg/m^2	29.4 (5.1)	30.1 (4.9)	30.8 (5.5)	27.0 (4.0)	27.6 (4.1)	5.9e-30
Statin use, n(%)	990 (66)	401 (88)	321 (61)	97 (36)	171 (70)	4.2e-45
Anti-hypertensive treatment use, n(%)	1072 (72)	419 (92)	352 (67)	129 (48)	172 (70)	8.4e-37
HbA1c, mmol/mol	51.4 (14.3)	58.7 (13 8)	56.8 (13.4)	38.7 (3.9)	40.0 (4.8)	2.0e-171
SBP, mmHg	136 (18.2)	138 (20 0)	136 (17 6)	132 (17.2)	134 (16.0)	7.2e-05
DBP, mmHg	77 (9.8)	76 (10.3)	78 (9.8)	77 (9.4)	77 (9.2)	0.0010
Pulse pressure, mmHg	58.7 (14.5)	62.6 (16.2)	58.2 (13.1)	54.5 (12.5)	57.2 (14.2)	5.6e-11
Tot Cholesterol, mmol/l	4.37 (1.1)	3.92 (0.9)	4.38 (1.0)	5.03 (1.1)	4.47 (1.1)	1.4e-40
HDL, mmol/l	1.33 (0.40)	1.19 (0.35)	1.32 (0.38)	1.51 (0.44)	1.42 (0.40)	3.9e-25
LDL, mmol/l	2.43 (0.92)	2.06 (0.74)	2.42 (0.91)	3.01 (0.94)	2.52 (0.88)	8.6e-37
Triglycerides, mmol/l	1.54 (0.86)	1.68 (0.94)	1.62 (0.92)	1.30 (0.65)	1.37 (0.70)	1.3e-10
Creatinine, serum, μmol/l	84.7 (24.8)	93.4 (32.2)	80.0 (20.6)	81.3 (18.5)	83.3 (19.3)	6.4e-13
ACR, mg/mmol	5.46 (25.6)	9.48 (37.4)	4.14 (16.5)	3.23 (22.7)	3.38 (15.3)	1.52e-16
eGFR	67.2 (19.8)	60.8 (20.3)	71.3 (19.4)	69.6 (17.7)	66.9 (18.9)	8.78e-14

Binary variables are reported as n(%) and quantitative data are reported as mean (SD). Differences between groups were tested by chi2 or Kruskal-Wallis tests, as appropriate.

Abbreviations used in this table:

BMI stands for Body Mass Index.

SBP and DBP stand for Systolic and Diastolic Blood Pressure, respectively.

HDL and LDL stand for High-Density and Low-density Lipoprotein, respectively.

ACR stands for albumin creatinine ratio.

eGFR stands for estimated Glomerular Filtration Rate.

Table 2. CVD in individuals with manifest T2D. Univariate comparisons.

Phenotype	**CVD in T2D** median (IQR)	**N**	**Non-CVD in T2D** median (IQR)	**N**	**p-value**
Male sex, n(%)	331 (72)	458	324 (61)	527	3.4e-04
Age, years	69 (64 - 75)	457	67 (61 - 73)	527	3.4e-07
T2D Duration, years	11 (6 – 16)	444	8 (4 – 12.2)	520	3.4e-09
BMI, kg/m^2	29.64 (26.9 - 32.9)	458	30.2 (26.79 - 34.16)	525	0.117
Statin use, n(%)	401 (88)	456	321 (61)	525	2.2e-21
Anti-hypertensive treatment use, n(%)	419 (92)	456	352 (67)	524	4.5e-21
HbA1c, mmol/mol	56 (49.73 - 66)	443	55 (48 - 63)	517	0.025
SBP, mmHg	136 (123 - 150)	451	135 (124 - 147)	524	0.245
DBP, mmHg	75 (68 - 82)	452	78 (71 - 84)	524	7.8e-05
Pulse pressure, mmHg	60 (51 – 72)	451	57 (49 – 66)	524	1.0e-04
Tot Cholesterol, mmol/l	3.8 (3.34 - 4.3)	447	4.3 (3.7 - 5)	515	5.4e-14
HDL, mmol/l	1.13 (0.93 - 1.4)	446	1.28 (1.06 - 1.5)	507	2.4e-08
LDL, mmol/l	1.97 (1.57 - 2.46)	429	2.3 (1.77 - 2.97)	488	3.8e-09
Triglycerides, mmol/l	1.43 (1.06 - 2.02)	443	1.35 (1 - 1.99)	506	0.150
Creatinine, serum, μmol/l	85 (73.37 - 101)	442	77.9 (66 - 91)	514	1.2e-10
ACR, mg/mmol	1.24 (0.60 – 3.60)	358	0.90 (0.51 – 1.90)	446	3.2e-5
eGFR, mL/min per 1.73 m2	58.4 (46.7 – 78.8)	428	75.2 (54.8 – 86.3)	510	7.0e-14
ABPI Right	1.11 (0.97 - 1.21)	434	1.15 (1.07 - 1.23)	507	2.5e-09
ABPI Left	1.11 (0.96 - 1.2)	430	1.15 (1.07 - 1.23)	504	1.3e-07
RHI	1.98(1.68 - 2.44)	420	2.07 (1.76 - 2.53)	492	0.036
PWV, m/s	11.15 (9.4 - 13.45)	343	10.7 (9 - 12.9)	433	6.2e-03
Common carotid IMT Right, mm	0.91 (0.8 - 1.07)	405	0.87 (0.75 - 0.99)	498	1.7e-05
Common carotid IMT Left, mm	0.93 (0.81 - 1.08)	404	0.89 (0.76 - 1.03)	492	1.4e-03
Carotid bulb Right, mm	1.17 (0.97 - 1.63)	342	1.03 (0.87 - 1.25)	448	2.0e-11
Carotid bulb Left, mm	1.16 (0.96 - 1.49)	350	1.06 (0.87 - 1.28)	443	1.8e-06

Binary variables are reported as n(%). Differences between groups were tested by chi2 or Mann–Whitney U tests, as appropriate.

Abbreviations used in this table:
BMI stands for Body Mass Index.
SBP and DBP stand for Systolic and Diastolic Blood Pressure, respectively.
HDL and LDL stand for High-Density and Low-density Lipoprotein, respectively.
ABPI stands for Ankle Brachial Index.
RHI stands for Reactive Hyperaemia Index.
PWV stands for Pulse Wave Velocity.
IMT stands for Intima Media Thickness.
ACR stands for albumin creatinine ratio.
eGFR stands for estimated Glomerular Filtration Rate.

Table 3. Logistic regression against CVD status in individuals with manifest T2D

Phenotype	Minimal model		Full model	
	OR (95%CI)	p-value	OR (95%CI)	p-value
SBP, mmHg	1.00 (0.99 - 1.01)	0.98	1.00 (0.99 - 1.01)	0.76
DBP, mmHg	0.97 (0.95 - 0.99)	5.9e-04	0.97 (0.95 - 0.99)	5.5e-03
ABPI Right	0.08 (0.03 - 0.20)	4.5e-08	0.09 (0.03 - 0.23)	1.6e-06
ABPI Left	0.15 (0.06 - 0.35)	9.3e-06	0.19 (0.08 - 0.48)	4.5e-04
RHI	0.84 (0.65 - 1.07)	0.16	0.89 (0.67 - 1.19)	0.43
PWV, m/s	1.01 (0.95 - 1.08)	0.75	1.00 (0.92 - 1.08)	0.89
CCA IMT Right, mm	2.59 (1.28 - 5.24)	8.0e-03	2.21 (1.00 - 4.88)	0.049
CCA IMT Left, mm	1.16 (0.63 - 2.16)	0.63	1.01 (0.51 - 2.03)	0.97
Bulb Right, mm	2.24 (1.59 - 3.15)	3.7e-06	1.84 (1.27 - 2.67)	1.2e-03
Bulb Left, mm	1.69 (1.14 - 2.5)	8.9e-03	1.52 (0.98 - 2.36)	0.061
eGFR, mL/min per 1.73 m2	0.98 (0.97 – 0.99)	7.1e-06	0.98 (0.97 – 0.99)	5.5e-05

The minimal model is a logistic regression adjusted for age, gender, statin use, anti-hypertensive treatment and current smoking.. The full model also includes adjustments for BMI, HDL, serum creatinine, HbA1c, diabetes duration and SBP (SBP not included in blood pressure analyses and serum creatinine not included in eGFR models).

Abbreviations used in this table:
SBP and DBP stand for Systolic and Diastolic Blood Pressure, respectively.
ABPI stands for Ankle Brachial Index
RHI stands for Reactive Hyperaemia Index.
PWV stands for Pulse Wave Velocity.
CCA stands for common carotid artery
IMT stands for Intima Media Thickness.
BMI stands for Body Mass Index.
HDL stands for High-Density Lipoprotein.
eGFR stands for estimated Glomerular Filtration Rate.

Table 4. T2D vs Non-T2D univariate comparisons

Phenotype	**T2D** median (IQR)	**N**	**Non-T2D** median (IQR)	**N**	**p-value**
Male sex, n(%)	655 (66)	985	324 (63)	515	0.166
Age, years	68 (63 - 74)	984	68 (62 - 74)	514	0.39
BMI, kg/m^2	29.9 (26.8 - 33.5)	983	26.8 (24.5 - 29.4)	512	3.2e-31
Statin use, n(%)	722 (74)	981	268 (52)	511	2.3e-16
Anti-hypertensive treatment use, n(%)	771 (79)	980	301 (59)	513	3.5e-16
HbA1c, mmol/mol	55.19 (48.63 - 65)	960	39 (37 - 42)	506	4.4e-173
SBP, mmHg	135 (124 - 148)	975	130 (121 - 144)	509	2.8e-05
DBP, mmHg	76 (69.5 - 83)	976	76 (70 - 82)	510	0.88
Pulse pressure, mmHg	58 (50 – 68)	975	54 (46 – 64)	513	1.1e-08
Tot Cholesterol, mmol/l	4 (3.5 - 4.76)	962	4.7 (3.92 - 5.56)	504	6.8e-24
HDL, mmol/l	1.22 (0.99 - 1.44)	953	1.40 (1.14 - 1.74)	500	3.6e-20
LDL, mmol/l	2.10 (1.64 - 2.74)	917	2.70 (2.03 - 3.4)	484	1.4e-24
Triglycerides, mmol/l	1.4 (1.01 - 2)	949	1.19 (0.88 - 1.61)	500	1.0e-11
Creatinine, serum, μmol/l	81.0 (69.0 - 94.8)	956	80.4 (70.0 - 91.1)	503	0.31
ACR, mg/mmol	1.00 (0.60 – 2.50)	804	0.68 (0.40 – 1.20)	388	1.2e-14
eGFR, mL/min per 1.73 m2	64.0 (51.0 – 83.8)	938	66.3 (54.8 – 82.9)	503	0.136
ABPI Right	1.14 (1.04 - 1.22)	941	1.14 (1.06 - 1.22)	497	0.52
ABPI Left	1.13 (1.03 - 1.22)	934	1.14 (1.05 - 1.21)	498	0.40
RHI	2.03 (1.73 - 2.50)	912	2.30 (1.90 - 2.75)	487	3.4e-10
PWV, m/s	10.9 (9.24 - 13.2)	776	9.66 (8.27 - 11.38)	452	8.7e-16
Common carotid IMT Right, mm	0.89 (0.77 - 1.03)	903	0.84 (0.74 - 0.98)	475	6.6e-05
Common carotid IMT Left, mm	0.91 (0.78 - 1.05)	896	0.86 (0.74 - 1.03)	475	1.9e-04
Carotid bulb Right, mm	1.08 (0.9 - 1.39)	790	1.02 (0.87 - 1.31)	430	5.5e-03
Carotid bulb Left, mm	1.09 (0.91 - 1.36)	793	1.06 (0.89 - 1.29)	431	0.037

Binary variables are reported as n(%). Differences between groups were tested by chi2 or Mann–Whitney U tests, as appropriate.

Abbreviations used in this table:
BMI stands for Body Mass Index.
SBP and DBP stand for Systolic and Diastolic Blood Pressure, respectively.
HDL and LDL stand for High-Density and Low-density Lipoprotein, respectively.
ABPI stands for Ankle Brachial Index
RHI stands for Reactive Hyperaemia Index.
PWV stands for Pulse Wave Velocity.
IMT stands for Intima Media Thickness.
ACR stands for albumin creatinine ratio.
eGFR stands for estimated Glomerular Filtration Rate.

Table 5. Logistic regression against T2D status, irrespective of CVD status

Phenotype	Minimal model		Full model	
	OR (95%CI)	p-value	OR (95%CI)	p-value
SBP, mmHg	1.01 (1.01 - 1.02)	9.0e-05	1.01 (0.99 - 1.02)	0.35
DBP, mmHg	1.00 (0.99 - 1.02)	0.83	0.99 (0.97 - 1.01)	0.44
ABPI Right	1.14 (0.57 - 2.27)	0.72	1.90 (0.49 - 7.3)	0.35
ABPI Left	0.83 (0.41 - 1.67)	0.60	1.17 (0.28 - 4.79)	0.83
RHI	0.63 (0.52 - 0.76)	2.1e-06	0.94 (0.67 - 1.32)	0.72
PWV, m/s	1.26 (1.18 - 1.34)	3.9e-13	1.07 (0.95 - 1.12)	0.25
CCA IMT Right, mm	2.21 (1.23 - 3.99)	0.0084	1.28 (0.46 - 3.55)	0.64
CCA IMT Left, mm	1.77 (1.04 - 3.01)	0.036	1.11 (0.46 - 2.67)	0.82
Bulb Right, mm	1.27 (0.97 - 1.65)	0.080	1.02 (0.65 - 1.61)	0.93
Bulb Left, mm	1.31 (0.94 - 1.84)	0.11	1.04 (0.59 - 1.85)	0.89
eGFR, mL/min per 1.73 m2	0.997 (0.99 – 1.00)	0.38	1.00 (0.99 – 1.02)	0.57

The minimal model is a logistic regression adjusted for age, gender, statin use, anti-hypertensive treatment, current smoking and CVD status. The full model also includes adjustments for BMI, HDL, serum creatinine, HbA1c and SBP (SBP not included in blood pressure analyses and serum creatinine not included in eGFR models).

Abbreviations used in this table:
SBP and DBP stand for Systolic and Diastolic Blood Pressure, respectively.
ABPI stands for Ankle Brachial Index
RHI stands for Reactive Hyperaemia Index.
PWV stands for Pulse Wave Velocity.
CCA stands for common carotid artery
IMT stands for Intima Media Thickness.
BMI stands for Body Mass Index.
HDL stands for High-Density Lipoprotein.
eGFR stands for estimated Glomerular Filtration Rate.

Table 6. Spearman correlations between imaging variables in all participants

Phenotype		ABPI Right	ABPI Left	Bulb IMT Right	Bulb IMT Left	CCA IMT Right	CCA IMT Left	PWV	RHI
ABPI Right	rho	1	0.747	-0.137	-0.152	-0.117	-0.132	-0.117	-0.02
	p	-	2.44e-254	2.50e-06	1.50e-07	1.94e-05	1.36e-06	4.84e-05	0.459
ABPI Left	rho	0.747	1	-0.149	-0.164	-0.139	-0.14	-0.111	-0.004
	p	2.44e-254	-	3.08e-07	1.44e-08	3.99e-07	3.10e-07	1.21e-04	0.884
Bulb IMT Right	rho	-0.137	-0.149	1	0.509	0.441	0.345	0.231	-0.059
	p	2.50e-06	3.08e-07	-	3.24e-77	2.02e-59	4.37e-35	5.38e-14	0.046
Bulb IMT Left	rho	-0.152	-0.164	0.509	1	0.361	0.456	0.195	-0.08
	p	1.50e-07	1.44e-08	3.24e-77	-	9.90e-39	4.66e-64	2.01e-10	0.006
CCA IMT Right	rho	-0.117	-0.139	0.441	0.361	1	0.593	0.318	-0.059
	p	1.94e-05	3.99e-07	2.02e-59	9.90e-39	-	2.68e-130	2.53e-28	0.033
CCA IMT Left	rho	-0.132	-0.14	0.345	0.456	0.593	1	0.264	-0.054
	p	1.36e-06	3.10e-07	4.37e-35	4.66e-64	2.68e-130	-	1.32e-19	0.052
PWV	rho	-0.117	-0.111	0.231	0.195	0.318	0.264	1	-0.01
	p	4.84e-05	1.21e-04	5.38e-14	2.01e-10	2.53e-28	1.32e-19	-	0.735
RHI	rho	-0.02	-0.004	-0.059	-0.08	-0.059	-0.054	-0.01	1
	p	0.459	0.884	0.046	0.006	0.033	0.052	0.735	-

Abbreviations used:
ABPI stands for Ankle Brachial Index.
CCA stands for common carotid artery
IMT stands for Intima Media Thickness.
PWV stands for Pulse Wave Velocity.
RHI stands for Reactive Hyperaemia Index.

Table 7. Spearman correlations in all T2D individuals

Phenotype		ABPI Right	ABPI Left	Bulb IMT Right	Bulb IMT Left	CCA IMT Right	CCA IMT Left	PWV	RHI
Age	rho	-0.043	-0.022	0.303	0.269	0.283	0.212	0.337	0.022
	p	0.187	0.503	3.50E-18	1.25E-14	5.09E-18	1.47E-10	4.55E-22	0.511
BMI	rho	-0.031	-0.027	-0.057	-0.117	-0.039	-0.056	0.114	-0.118
	p	0.349	0.416	0.112	9.44E-04	0.239	0.092	0.001	3.59E-04
SBP	rho	-0.114	-0.113	0.089	0.121	0.152	0.144	0.461	0.152
	p	4.72E-04	5.24E-04	0.013	6.60E-04	4.96E-06	1.63E-05	4.91E-42	4.22E-06
DBP	rho	0.003	0.024	-0.155	-0.112	-0.055	-0.014	0.223	0.028
	p	0.921	0.462	1.25E-05	0.002	0.101	0.687	3.80E-10	0.403
HbA1c	rho	-0.039	-0.018	0.057	0.059	0.019	0.053	0.122	-0.06
	p	0.240	0.590	0.115	0.100	0.576	0.116	7.54E-04	0.072
Tot Chol	rho	-0.026	-0.026	-0.079	-0.054	0.02	0.007	-0.071	0.065
	p	0.435	0.427	0.028	0.131	0.557	0.843	0.051	0.051
LDL	rho	0.016	0.015	-0.052	-0.039	0.04	0.038	-0.052	0.057
	p	0.644	0.654	0.155	0.285	0.242	0.271	0.160	0.094
HDL	rho	0.041	0.044	-0.052	-0.055	-0.093	-0.106	-0.098	0.115
	p	0.214	0.189	0.149	0.127	0.006	0.002	0.007	5.39E-04
Triglycerides	rho	-0.067	-0.078	0.004	0.005	0.05	0.029	0.063	-0.1
	p	0.042	0.019	0.917	0.890	0.136	0.389	0.085	0.003
Creatinine	rho	-0.004	-0.036	0.168	0.147	0.131	0.117	0.161	-0.043
	p	0.909	0.281	3.23E-06	4.80E-05	1.16E-04	5.84E-04	8.92E-06	0.204
eGFR	rho	0.01	0.036	-0.213	-0.183	-0.175	-0.135	-0.21	0.037
	p	0.755	0.285	3.13E-09	3.88E-07	2.16E-07	7.44E-05	6.11E-09	0.267
ACR	rho	-0.087	-0.117	0.12	0.122	0.088	0.03	0.248	-0.064
	p	0.016	0.001	0.002	0.002	0.015	0.416	1.90E-10	0.078
T2D duration	rho	-0.053	-0.062	0.185	0.164	0.060	0.028	0.186	-0.047
	p	0.106	0.063	2.30e-7	4.40e-6	0.073	0.406	2.27e-7	0.138

Table 8. Spearman correlations in all Non-T2D individuals

Phenotype		ABPI Right	ABPI Left	Bulb IMT Right	Bulb IMT Left	CCA IMT Right	CCA IMT Left	PWV	RHI
Age	rho	-0.052	-0.045	0.428	0.368	0.458	0.305	0.576	0.002
	p	0.247	0.311	1.49E-20	2.79E-15	4.59E-26	1.06E-11	2.60E-41	0.964
BMI	rho	0.041	0.037	0.094	0.015	0.1	0.117	0.193	-0.263
	p	0.362	0.406	0.052	0.751	0.030	0.011	3.56E-05	3.67E-09
SBP	rho	-0.083	-0.098	0.274	0.217	0.338	0.303	0.45	0.106
	p	0.063	0.028	9.12E-09	5.47E-06	4.92E-14	1.84E-11	7.20E-24	0.019
DBP	rho	0.029	-0.013	-0.062	-0.089	-0.036	0.056	0.086	0.069
	p	0.513	0.777	0.200	0.067	0.430	0.226	0.069	0.128
HbA1c	rho	-0.072	-0.131	0.142	0.13	0.121	0.088	0.12	-0.059
	p	0.112	0.004	0.004	0.007	0.009	0.057	0.012	0.194
Tot Chol	rho	-0.046	0.024	-0.114	-0.152	-0.074	-0.032	-0.074	0.171
	p	0.314	0.604	0.019	0.002	0.108	0.489	0.120	1.71E-04
LDL	rho	-0.002	0.059	-0.055	-0.1	-0.033	0.017	-0.055	0.097
	p	0.962	0.204	0.265	0.044	0.488	0.727	0.258	0.037
HDL	rho	-0.066	-0.02	-0.115	-0.123	-0.123	-0.138	-0.135	0.238
	p	0.148	0.665	0.018	0.011	0.008	0.003	0.004	1.56E-07
Triglycerides	rho	-0.004	0.005	0.021	-0.013	0.077	0.11	0.089	-0.134
	p	0.934	0.907	0.662	0.794	0.100	0.018	0.061	0.004
Creatinine	rho	0.136	0.098	0.132	0.143	0.174	0.119	0.168	-0.138
	p	0.003	0.031	0.007	0.003	1.62E-04	0.010	3.77E-04	0.002
eGFR	rho	-0.097	-0.074	-0.217	-0.199	-0.266	-0.179	-0.289	0.113
	p	0.033	0.103	6.54E-06	3.62E-05	5.61E-09	9.64E-05	5.44E-10	0.013
ACR	rho	-0.158	-0.087	0.089	0.145	0.226	0.143	0.172	-0.081
	p	0.002	0.092	0.100	0.007	9.46E-06	0.006	0.001	0.123

Abbreviations used in tables 7 and 8:
BMI stands for Body Mass Index.
SBP and DBP stand for Systolic and Diastolic Blood Pressure, respectively.
HDL and LDL stand for High-Density and Low-density Lipoprotein, respectively.
ABPI stands for Ankle Brachial Index.
CCA stands for common carotid artery
IMT stands for Intima Media Thickness.
PWV stands for Pulse Wave Velocity.
RHI stands for Reactive Hyperaemia Index.
eGFR stands for estimated Glomerular Filtration Rate
ACR stands for albumin/creatinine ratio

Supplemental table 1. CVD in individuals without manifest T2D. Univariate comparisons.

Phenotype	**CVD in NonT2D** median (IQR)	**N**	**Non-CVD in NonT2D** median (IQR)	**N**	**p-value**
Male sex, n(%)	161 (65.7)	245	165 (60.4)	273	0.215
Age, years	68 (63 - 75)	245	68 (61 - 73)	272	0.059
BMI, kg/m^2	27.1 (24.8 - 30.02)	243	26.6 (24.25 - 29.39)	273	0.118
Statin use, n(%)	171 (69.8)	245	100 (37.2)	269	1.4e-15
Anti-hypertensive treatment use, n(%)	172 (70.2)	245	131 (48.3)	271	4.7e-7
HbA1c, mmol/mol	39 (37.0 – 42.0)	239	39 (36.6 – 41.0)	267	0.016
SBP, mmHg	133 (124 - 144)	243	129 (120 - 144)	270	0.062
DBP, mmHg	76 (70 - 83)	243	77 (70 - 82)	271	0.872
Pulse pressure, mmHg	55 (47 – 64)	243	53 (45 – 64)	270	0.042
Tot Cholesterol, mmol/l	4.3 (3.7 - 5)	237	5 (4.22 - 5.79)	267	1.9e-9
HDL, mmol/l	1.38 (1.1 - 1.68)	235	1.4 (1.17 - 1.81)	265	0.07
LDL, mmol/l	2.42 (1.9 - 3.07)	227	2.97 (2.34 - 3.64)	257	8.0e-10
Triglycerides, mmol/l	1.19 (0.89 - 1.7)	236	1.2 (0.88 - 1.54)	264	0.529
Creatinin, serum, μmol/l	82 (70 - 93)	237	79 (69 - 89)	266	0.145
ACR, mg/mmol	0.7 (0.42 - 1.4)	168	0.6 (0.4 - 1)	220	0.212
eGFR, mL/min per 1.73 m2	63.2 (53.1 - 82.5)	237	68.8 (56.0 - 83.1)	266	0.068
ABPI Right	1.13 (1.03 - 1.22)	237	1.14 (1.08 - 1.23)	264	0.172
ABPI Left	1.14 (1.04 - 1.2)	237	1.14 (1.06 - 1.22)	265	0.327
RHI	2.27 (1.82 - 2.71)	229	2.34 (1.94 - 2.8)	259	0.076
PWV, m/s	9.93 (8.47 - 11.57)	209	9.47 (8.15 - 11.1)	243	0.083
Common carotid IMT Right, mm	0.87 (0.75 - 0.99)	219	0.83 (0.73 - 0.96)	256	0.101
Common carotid IMT Left, mm	0.87 (0.75 - 1.04)	220	0.85 (0.71 - 1.01)	255	0.106
Carotid bulb Right, mm	1.06 (0.88 - 1.36)	202	1.00 (0.86 - 1.23)	228	0.019
Carotid bulb Left, mm	1.10 (0.93 - 1.37)	203	1.02 (0.86 - 1.24)	228	0.0028

Binary variables are reported as n(%). Differences between groups were tested by chi2 or Mann–Whitney U tests, as appropriate.

Abbreviations used in this table:
BMI stands for Body Mass Index.
SBP and DBP stand for Systolic and Diastolic Blood Pressure, respectively.
HDL and LDL stand for High-Density and Low-density Lipoprotein, respectively.
ABPI stands for Ankle Brachial Index.
RHI stands for Reactive Hyperaemia Index.
PWV stands for Pulse Wave Velocity.
IMT stands for Intima Media Thickness.
ACR stands for albumin creatinine ratio.
eGFR stands for estimated Glomerular Filtration Rate.

Supplemental table 2. Backward linear regressions in T2D only

Dependent phenotype	Independent phenotype	Beta	SE	p-value
CCA IMT Right*				
	Age	0.066	0.010	1.6e-10
	Gender	-0.050	0.018	0.0055
	LDL	0.020	0.0089	0.021
	SBP	0.047	0.011	3.9e-5
	DBP	-0.059	0.012	9.5e-7
CCA IMT Left*				
	Age	0.055	0.011	2.7e-7
	Gender	-0.050	0.019	0.0074
	HDL	-0.021	0.0086	0.016
	SBP	0.047	0.012	1.2e-4
	DBP	-0.035	0.013	0.0048
Bulb IMT Right*				
	Age	0.048	0.016	0.0022
	Gender	-0.065	0.028	0.020
	T2D duration	0.034	0.013	0.0083
	CVD status	0.111	0.027	4.2e-5
	Triglycerides	0.037	0.015	0.015
	SBP	0.068	0.019	3.7e-4
	DBP	-0.050	0.020	0.011
Bulb IMT Left*				
	Gender	-0.068	0.026	0.0083
	LDL	0.039	0.014	0.0067
	SBP	0.073	0.018	4.8e-5
	DBP	-0.045	0.018	0.011
ABPI Right				
	Gender	-0.047	0.015	0.0016
	CVD status	-0.085	0.014	2.2e-9
	Current smoking	-0.101	0.022	3.3e-6
	SBP	-0.048	0.0098	1.0e-6
	DBP	0.031	0.0097	0.0013
ABPI Left				
	Gender	-0.054	0.009	5.1e-4
	CVD status	-0.054	0.014	1.2e-4
	Current smoking	-0.087	0.021	4.5e-5
	SBP	-0.051	0.0098	2.2e-7
	DBP	0.038	0.0096	7.1e-5
	HbA1c	0.019	0.0076	0.013

Supplemental table 2. Continued

Dependent phenotype	Independent phenotype	Beta	SE	p-value
Pulse wave velocity*	Age	0.091	0.0096	5.1e-20
	Gender	-0.039	0.017	0.027
	BMI	0.053	0.0098	9.4e-8
	LDL	-0.022	0.0087	0.011
	SBP	0.092	0.0088	2.9e-23
	HbA1c	0.025	0.0083	0.0026
RHI*	Gender	0.061	0.021	0.0035
	BMI	-0.038	0.011	5.9e-4
	SBP	0.084	0.013	5.0e-10
	DBP	-0.041	0.014	0.0036
	Anti-hypertensive medication	-0.069	0.024	0.0041

*Variable was log transformed.

Backward linear regressions. Candidate variables with $p<0.1$ were retained in the models. Only covariates that remain in the final models with $p<0.05$ are reported in this table. The following candidate variables were entered into the backward regression: Age, Gender, BMI, HbA1c, eGFR, T2D duration, Current smoker, Statin use, Antihypertensive medication use, SBP, DBP, Triglycerides, HDL, LDL, CVD status, RHI and 3 centre variables. Continuous variables were standardized to mean=0 and std=1.