Measures of atherosclerotic burden are associated with clinically manifest cardiovascular disease in type 2 diabetes: a European cross-sectional study

Shore, A. C.; Colhoun, H. M.; Natali, A.; Palombo, C.; Östling, Gerd; Aizawa, K.; Kennback, C.; Casanova, F.; Persson, Margaretha; Gooding, K.; Gates, P. E.; Khan, F.; Looker, H. C.; Adams, F.; Belch, J.; Pinnoli, S.; Venturi, E.; Morizzo, C.; Goncalves, Isabel; Ladenvall, Claes; Nilsson, Jan

Published in:
Journal of Internal Medicine

DOI:
10.1111/joim.12359

2015

Document Version:
Peer reviewed version (aka post-print)

R2

Measures of atherosclerotic burden are associated with clinically manifest cardiovascular disease in type 2 diabetes: a European cross-sectional study

Angela C. Shore[1], Helen M Colhoun[2], Andrea Natali[3], Carlo Palombo[4], Gerd Östling[5], Kunihiko Aizawa[1], Cecilia Kennbäck[5], Francesco Casanova[1], Margaretha Persson[5], Kim Gooding[1], Phillip E. Gates[1], Faisel Kahn[2], Helen C Looker[2], Fiona Adams[2], Jill Belch[2], Silvia Pinnoli[3], Elena Venturi[3], Carmela Morizzo[4], Isabel Goncalves[5], Claes Ladenvall[5], Jan Nilsson[5] on behalf of the SUMMIT consortium.

[1]Diabetes and Vascular Medicine, University of Exeter Medical School and NIHR Exeter Clinical Research Facility, Exeter, [2]Medical Research Institute, University of Dundee, Dundee, UK, [3]Department of Clinical and Experimental Medicine and [4]Department of Surgical, Medical, Molecular and Critical Area Pathology, University of Pisa, Italy and [5]Department of Clinical Sciences Malmö, Lund University, Sweden

Running title: Vascular changes in T2D and CVD

Correspondence to:

Jan Nilsson

CRC 91:12, Jan Waldenströms gata 35, Skåne University Hospital, S-205 02 Malmö, Sweden

Phone: +46 40 39 12 30, Fax: +46 40 39 12 12

Email: Jan.Nilsson@med.lu.se

Subject codes: 190 type 2 diabetes, 135 risk factors (atherosclerosis), 61 other imaging, 33 other diagnostic testing

Abstract

Background – There is a need to develop and validate surrogate markers of cardiovascular disease (CVD) in diabetes. The macrovascular changes associated with diabetes include aggravated atherosclerosis, increased arterial stiffness and endothelial dysfunction. In the present study our aim was to determine which of these factors most associated with clinically manifest cardiovascular event.

Methods and results – We measured vascular changes 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 intima media thickness (IMT) and ankle-brachial pressure index were independently associated with the presence of CVD in T2D, while pulse wave velocity and endothelial function provided limited independent additive information. Measurement of IMT in the carotid bulb provided a better discrimination of the presence of CVD in T2D than measurement of IMT in the common carotid artery. The factors most significantly associated with increased carotid IMT in T2D were age, diabetes duration, systolic blood pressure, impaired renal function and increased arterial stiffness, whereas no or weak independent associations were found with metabolic factors and endothelial dysfunction.

Conclusions - Measures of atherosclerotic burden are associated with clinically manifest CVD in T2D. In addition, vascular changes that are not directly related to known metabolic risk factors are important in atherosclerosis and CVD in T2D. A better understanding of the mechanisms involved is crucial for enabling better identification of CV risk in diabetes.

Key words: cardiovascular disease, diabetes mellitus, risk factors, vasculature

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 who have had diabetes for >10 years, with some studies reporting risks equivalent to that of a non-diabetic person who has had a previous MI [2]. Diabetes is also associated with an increased risk of stroke and lower extremity arterial disease (LEAD), as well as an increased risk of complications after acute CVD events, and of recurrent CVD events [1].

Prevention of cardiovascular (CV) complications in diabetes represents an increasingly important medical and socioeconomic challenge. Intensive glycemic control provides protection against development of microvascular complications, such as diabetic retinopathy, nephropathy and neuropathy, in diabetes and macrovascular disease in type 1 diabetes but the effect on macrovascular complications in type 2 diabetes (T2D) is less clear [4-6]. New generations of anti-diabetic therapies, are being evaluated for their ability to reduce CVD in T2D, but have so far failed to demonstrate significant protective effects [7-9]. Observations from long-term follow-up of patients suggest that a lowering of CV risk only becomes evident after several years of intensive 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. CV risk prediction in diabetes is today primarily based on determining HbA1c levels and monitoring of traditional CV risk factors, while there is limited evidence for the value of vascular measurements.

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 be used to monitor response to therapy. The primary aim of the present SUMMIT cross-sectional study, carried out at four European centers, was to identify vascular changes associated with clinically manifest CVD in T2D. The intima-media media thickness (IMT) of the common carotid arteries (CCA) and the carotid bulbs was used as a surrogate marker of carotid atherosclerosis and ankle-brachial pressure index (ABPI) as a surrogate marker of lower extremity arterial disease (LEAD). Pulse wave velocity (PWV) was used to determine arterial stiffness and endothelial function assessed by the reactive hyperemia index (RHI). All of these measures have previously been related to CVD risk in general population studies. Our observations suggest that vascular

changes associated with CVD in T2D are best assessed by measuring ABPI and IMT in the right carotid artery.

Methods

Study population

The study cohort of consisted of 4 groups; (1) subjects with T2D and clinically manifest CVD, (2) subjects with T2D but without clinical signs of CVD, (3) subjects with CVD but no diabetes and (4) subjects without both CVD and diabetes recruited from existing population cohorts and hospital registers at the university hospitals in Malmö (Sweden), Pisa (Italy), and Dundee and Exeter (UK) between December 2010 and April 2013. Diabetes was defined on the basis of contemporary or historical evidence of hyperglycemia (according to WHO 1998 criteria; fasting plasma glucose >7.0 mmol/l or 2-h plasma glucose >11.1mmol/l, or both) or by current medication with insulin, sulphonylureas, metformin or other anti-diabetic drugs. To define T2D, we required that patients should have been diagnosed after the age of 30. Subjects diagnosed with T2D < 35 years of age or treated with insulin within12 months of diagnosis were not included the study. Classification of CVD included non-fatal acute MI, hospitalized unstable angina, resuscitated cardiac arrest, any coronary revascularization procedure, non-fatal stroke, transient ischemic attack confirmed by a specialist, 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 at each center for gender, age (± 5 years) and duration of diabetes (± 5 years). Subjects without T2D were matched for gender and age (± 5 years) at each center. Subjects with CVD with or without T2D were matched for CVD type. Exclusion criteria included 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. Demographics, clinical characteristics including medication, physical and laboratory examinations were obtained according to a pre-defined study protocol at all 4 participating centers. The study was approved by the local ethical review boards and all study subjects provided written informed consent and was carried out in accordance with the principles of the Declaration of Helsinki.

Ultrasound imaging for IMT

An ultrasound examination of the carotid arteries was performed to assess atherosclerotic status. All centres used existing ultrasound devices that they were accustomed to work with. The subject was in a supine position with the head turned approximately 45 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 curve. If a plaque was present it was included in the IMT measurement. The sonographer took the images striving to get the echoes representing the transitions between lumen and intima and media and adventitia in the far wall sharp over at least 10 mm of the CCA and bulb had 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 of the artery at each site and measured 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 the lumen–intima and media–adventitia transitions. The echoes were automatically outlined in the analysis system, with the possibility for the observer to make manual adjustments when needed. The computer system measured the distance between the lines at approximately 100 sites over each 10 mm section, and values for the 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 to the beginning of the bulb.

Before the start of the study sonographers from the four centres participated in an assessment of interobserver variability. Images were taken for measurement of IMT in CCA and in the bulb using the same machine. Images from 17 subjects were examined by a sonographer from each centre. The examinations were performed without any knowledge of the findings from the other sonographers. The absolute difference between centers for IMT in CCA was 0.089 mm (10.1 %), range 0.07 – 0.11 mm (intra-class correlation coefficient 0.84 [95% C.I. 0.72;0.91]) and for IMT bulb it was 0.17 mm (14.1 %), range 0.10 – 0.24 mm (intra-class correlation coefficient 0.90 [95% C.I. 0.81;0.95]). Further training to decrease inter-observer variability was performed throughout the months preceding the start of the study resulting in an inter-observer variation of less than 5% (intra-class correlation coefficient 0.97 [95% C.I.

0.93;0.98]) for the CCA and less than 10% (intra-class correlation coefficient 0.97 [95% C.I. 0.88;0.99]) for the bulb.

Measurements of endothelial function and arterial stiffness

The subjects were asked to refrain from coffee and tea for ≥2 h, nicotine for ≥4 h and alcohol intake for ≥12 hours before the investigation. Only a light meal was allowed during the previous 3 hours. The examination was performed in a quiet room, at 21–24°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) to estimate the endothelium-dependent vasodilation following post-ischemic hyperemia. 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 mins 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 5 mins 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, was recorded for another 8 mins. The 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" (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) or "NonStandOccLen" (time of occlusion was > or < 5 mins).

Arterial stiffness was assessed by calculating PWV using a Sphygmocor device (Atcor Medical, Australia). A blood pressure cuff was attached to the left arm, and three electrocardiographic electrodes (lead I) were attached. The carotid and femoral pulses were carefully located. The proximal distance was measured as from the carotid pulse to the fossa jugularis. The distal distance was entered as the combined distances of fossa jugularis to umbilicum, and umbilicum to femoral pulse. PWV equalled the distal minus proximal distance. After 5 mins of rest the blood pressure was measured three times, with 1 min between measurements, and the mean value of the two final measurements was entered. The carotid and femoral pulses were captured. 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 divided by the calculated distance.

Ankle brachial pressure index

Blood pressure cuffs of the size appropriate for the subject 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. The 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 clinical characteristics between the groups with or without CVD and with or without T2D 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 the groups with or without CVD and with or without T2D. The first model (minimal model) adjusted for age, gender, statin use, anti-hypertensive treatment and current smoking. The second model (full model) adjusted for the same characteristics plus the following anthropometric and metabolic factors characteristic for T2D: body mass index (BMI), high density lipoprotein (HDL), serum creatinine, HbA1c and SBP (SBP not included in blood pressure analyses and serum creatinine not included in estimated glomerular filtration rate (eGFR) models). The comparisons between the T2D and Non-T2D groups were also adjusted for CVD status, and the comparisons between the CVD and Non-CVD in T2D groups for diabetes duration. Differences in CVD risk effect estimates between the T2D and Non-T2D groups were evaluated by incorporating an interaction term between T2D status and phenotype. Interaction effects were evaluated in the full logistic regression model, excluding HbA1c and serum creatinine. The logistic regressions were 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 the recruitment center was included as a covariate. Reported p-values are nominal. Because 11 imaging variables were tested across the four different groups of subjects we considered p-values below 0.05/44 = 0.0011 to be significant in the logistic regressions. Analyses were done using the R version 2.15.2 software package. All statistical analyses were done in accordance with the original protocol of the study.

Results

The study cohort included 458 subjects with T2D and clinically manifest CVD, 527 with T2D but without clinical signs of CVD, 245 with CVD but no diabetes and 270 without both CVD and T2D. The median age of the study population was 67.5 years and 979 (65%) were male. The clinical characteristics of the study cohort are listed in supplemental table 1.

Vascular changes associated with CVD in subjects with T2D

In the univariate analysis, T2D patients with CVD were older, had a longer duration of T2D, more often on statin and anti-hypertensive treatment, had more impaired renal function and lower levels of lipoprotein lipids (table 1). They also had a lower diastolic BP and ABPI, increased PVW as well as increased IMT both in the CCA and in the bulb. 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, statin and anti-hypertensive treatment in the minimal logistic regression model, ABPI (both right and left), carotid bulb IMT (both right and left), right CCA IMT, eGFR and diastolic BP remained significantly associated with clinically manifest CVD in T2D (table 2).

The associations between presence of CVD and ABPI, carotid bulb IMT, eGFR and diastolic blood pressure remained significant when also controlling for anthropometric and metabolic factors characteristic of T2D including HDL cholesterol, systolic blood pressure, BMI and HbA1c in the full logistic regression model (table 2). T2D patients with CVD had a lower

RHI in the minimal model, but this difference did not remain significant when in the full model (table 2). Thus, when controlling other CV risk factors only eGFR and surrogate markers of atherosclerosis such as ABPI and carotid IMT remained significantly associated with clinically manifest CVD in T2D.These observations reinforce the notion that CVD in T2D primarily is associated with more advanced atherosclerosis. Interestingly, these associations with CVD were markedly weaker in individuals without diabetes (supplemental table 2).

Vascular changes associated with T2D, irrespective of CVD

Subjects with T2D had increased IMT in the carotid bulb and the CCA, increased PWV and a lower RHI than those without T2D (table 3). 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. For the renal function factors, the albumin/creatinine ration (ACR) was higher in T2D subjects but the serum creatinine and eGFR were not increased. The difference between CCA IMT (left and right), systolic blood pressure, PWV and RHI in subjects with and without T2D remained significant in the minimal logistic regression model (table 4). However, all these associations became non-significant in the full linear regression model (table 4). Accordingly, the increased arterial stiffness and endothelial dysfunction in T2D appear to be clearly related to the metabolic changes.

Associations between different types of vascular changes

If the different types of vascular changes have a common etiology they would be likely to demonstrate a high degree of co-variation. To determine if this is the case we analyzed the correlations between carotid IMT, ABPI, PVW and endothelial dysfunction. Increased carotid bulb and CCA IMT correlated with a higher PWV and a lower ABPI in analysis of all study subjects (table 5). 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). The RHI showed a weak inverse

association with left carotid bulb IMT (table 5), otherwise the RHI did not demonstrate significant associations with vascular measurements reflecting atherosclerosis burden. Taken together, these observations demonstrate that increased arterial stiffness but not endothelial dysfunction are associated with measures of atherosclerosis.

Associations between CVD risk factors and vascular changes

The factors that contribute to development of different types of vascular changes in T2D remains to be fully elucidated. In the present study CCA and bulb IMT were found to correlate with age, systolic BP and impaired renal function in subjects with T2D (table 6). 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 using backward linear regression, while the association with impaired renal function was lost when adjusting for age, gender and other cardiovascular risk factors (supplemental table 3).

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 3).

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).

The ABPI demonstrated significant association with systolic BP and to some extent also with renal function. Diastolic BP correlated inversely with IMT in the carotid bulb but showed no association with CCA IMT (supplemental table 4). However, there was no significant association of ABPI with age and duration of diabetes (supplemental table 4). 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 6). 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 3).

Correlations between risk factors and vascular changes were generally the same among non-diabetic subjects as for those with T2D (supplemental table 5). However, unexpectedly, associations of carotid IMT with HbA1c and HDL seem to be stronger in non-diabetic individuals 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 [1]. In the present study we assessed how each of these changes related to the presence of clinically manifest CVD in T2D. We demonstrate that measurements of atherosclerotic burden such as 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. The lack of association between CVD and the assays measuring arterial stiffness and endothelial dysfunction are unexpected since these changes are considered to be important components of diabetic vasculopathy.

An additional novel observation of potential clinical importance is that measurement of IMT in the bulb may provide 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 mm 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.

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 in subjects with T2D is the main reason for the higher incidence of CVD in this disease. The most important determinants of increased carotid bulb IMT in T2D subjects were age, diabetes duration and high systolic BP. 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. Moreover, there was no association between carotid IMT and LDL in subjects with T2D and

this was true for both those treated with statins as well as those not treated with statins. 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 even stronger in this group, 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 in the development of atherosclerosis in T2D. In support of this, we found that 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. Consistent with our 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].

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 [18]. In a subanalysis of 4220 subjects with diabetes from an ongoing meta-analysis of 17 population-based cohorts den Ruijter and coworkers found no additive predictive value of CCA IMT to Framingham risk score [19]. 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.

A decreased eGFR was found to be independently associated with CVD in T2D in this study. Although we did not detect a significant difference in eGFR between CVD and non-CVD among non-T2D subjects in the present study it is well established that in the general population both an increased ACR and a decreased eGFR predict risk for cardiovascular death [20]. 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, a recent meta-analysis has shown that the relative cardiovascular risk of impaired renal function is the same irrespective of the presence or absence of diabetes [21]. Accordingly, although nephropathy is a common complication in diabetes it does not seem to be a diabetes-specific risk factor for CVD. In line with several previous studies [22, 23] we also found a correlation between decreased eGFR and increased carotid IMT

suggesting that impaired renal function may contribute to CVD by aggravating atherosclerosis. We unexpectedly observed independent inverse associations between diastolic BP and both CVD and carotid IMT in the present study. These associations could not be accounted for by more frequent anti-hypertensive treatment in the CVD group. The reasons for these associations remain to be fully understood.

Interestingly, the present study was not able to demonstrate independent association between CVD and measurements of arterial dysfunction and arterial stiffness. 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 when controlling for other risk factors. 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 BMI and low HDL have an important role in diabetic endothelial dysfunction. Although the present study does 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 than those without CVD, this difference did not remain significant when adjusting for age, gender, smoking, statin use and anti-hypertensive treatment. However, 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, this is not a prospective study. The cross-sectional design of the study does not allow conclusions regarding association of vascular changes in T2D with risk for future development of CVD. Hence, it cannot be excluded that the vascular changes observed in the T2D CVD group 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. Moreover, associations identified in cross-sectional studies do not prove causality. Standardization of carotid IMT measurements by ultrasound represents an important challenge in large cohort studies particularly when several centers and different type of equipment are involved. 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- and intra-observer variability. Identification of a history of cardiovascular events was based on hospital records and did not involve validation by independent experts. Accordingly, it cannot be excluded some subjects in the CVD groups may have been missed-diagnosed. It can also not be excluded that some subjects in the non-CVD groups could have had a history of a silent CV event.

Conclusion

We have shown that T2D subjects who have clinically manifest CVD are characterized by a more severe development of atherosclerosis as assessed by an increased carotid IMT and a lower ABPI. Our findings suggest that measuring ABPI and IMT in the carotid artery bulb could be used to assess vascular changes associated with CVD in T2D. Our observations also suggest that vascular changes not directly related to known metabolic risk factors are of importance for the development of atherosclerosis and CVD in T2D. Our findings could help to inform clinicians of the most suitable measure to use to monitor CVD in T2D, and may lead to better identification of risk for CV complications in diabetes. To further evaluate the ability these methods to predict CVD risk in T2D we will continue to monitor incident CV events in the present cohort during a 5-year period.

Funding Sources

This work was supported with funding from the Innovative Medicines Initiative (the SUMMIT consortium, IMI-2008/115006.

Acknowledgements

Elevate Scientific are acknowledged for helpful discussion when preparing the manuscript.

Disclosures

None

References

1 Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *JAMA* 2002; **287:** 2570-81.

2 Sattar N. Revisiting the links between glycaemia, diabetes and cardiovascular disease. *Diabetologia* 2013; **56:** 686-95.

3 Gregg EW, Cheng YJ, Saydah S, Cowie C, Garfield S, Geiss L, Barker L. Trends in death rates among U.S. adults with and without diabetes between 1997 and 2006: findings from the National Health Interview Survey. *Diabetes Care* 2012; **35:** 1252-7.

4 Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352:** 837-53.

5 Group AC, Patel A, MacMahon S*, et al.* Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *New England Journal of Medicine* 2008; **358:** 2560-72.

6 Ray KK, Seshasai SR, Wijesuriya S*, et al.* Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials. *Lancet* 2009; **373:** 1765-72.

7 Scirica BM, Bhatt DL, Braunwald E*, et al.* Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *The New England journal of medicine* 2013; **369:** 1317-26.

8 Lago RM, Singh PP, Nesto RW. Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. *Lancet* 2007; **370:** 1129-36.

9 Monami M, Ahren B, Dicembrini I, Mannucci E. Dipeptidyl peptidase-4 inhibitors and cardiovascular risk: a meta-analysis of randomized clinical trials. *Diabetes, obesity & metabolism* 2013; **15:** 112-20.

10 Nathan DM, Cleary PA, Backlund JY*, et al.* Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med* 2005; **353:** 2643-53.

11 Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *The New England journal of medicine* 2008; **359:** 1577-89.

12 Wendelhag I, Liang Q, Gustavsson T, Wikstrand J. A new automated computerized analyzing system simplifies readings and reduces the variability in ultrasound measurement of intima-media thickness. *Stroke; a journal of cerebral circulation* 1997; **28:** 2195-200.

13 Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX. Association of coronary heart disease incidence with carotid arterial wall thickness and major risk factors: the Atherosclerosis Risk in Communities (ARIC) Study, 1987-1993. *Am J Epidemiol* 1997; **146:** 483-94.

14 Hedblad B, Nilsson P, Janzon L, Berglund G. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmo, Sweden. *Diabet Med* 2000; **17:** 299-307.

15 Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 1997; **96:** 1432-7.

16 Polak JF, Person SD, Wei GS*, et al.* Segment-specific associations of carotid intima-media thickness with cardiovascular risk factors: the Coronary Artery Risk Development in Young Adults (CARDIA) study. *Stroke; a journal of cerebral circulation* 2010; **41:** 9-15.

17 Kinouchi M, Aihara KI, Fujinaka Y*, et al.* Diabetic Conditions Differentially Affect the Endothelial Function, Arterial Stiffness and Carotid Atherosclerosis. *Journal of atherosclerosis and thrombosis* 2014.

18 Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007; **115:** 459-67.

19 den Ruijter HM, Peters SA, Groenewegen KA*, et al.* Common carotid intima-media thickness does not add to Framingham risk score in individuals with diabetes mellitus: the USE-IMT initiative. *Diabetologia* 2013; **56:** 1494-502.
20 Chronic Kidney Disease Prognosis C, Matsushita K, van der Velde M*, et al.* Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet* 2010; **375:** 2073-81.
21 Fox CS, Matsushita K, Woodward M*, et al.* Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. *Lancet* 2012; **380:** 1662-73.
22 Kawamoto R, Ohtsuka N, Kusunoki T, Yorimitsu N. An association between the estimated glomerular filtration rate and carotid atherosclerosis. *Internal medicine* 2008; **47:** 391-8.
23 Desbien AM, Chonchol M, Gnahn H, Sander D. Kidney function and progression of carotid intima-media thickness in a community study. *American journal of kidney diseases : the official journal of the National Kidney Foundation* 2008; **51:** 584-93.
24 Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54:** 1615-25.
25 Rubinshtein R, Kuvin JT, Soffler M*, et al.* Assessment of endothelial function by non-invasive peripheral arterial tonometry predicts late cardiovascular adverse events. *European heart journal* 2010; **31:** 1142-8.
26 Lumsden NG, Andrews KL, Bobadilla M*, et al.* Endothelial dysfunction in patients with type 2 diabetes post acute coronary syndrome. *Diabetes & vascular disease research : official journal of the International Society of Diabetes and Vascular Disease* 2013; **10:** 368-74.
27 Hamburg NM, Keyes MJ, Larson MG*, et al.* Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008; **117:** 2467-74.
28 Schindler TH, Cadenas J, Facta AD*, et al.* Improvement in coronary endothelial function is independently associated with a slowed progression of coronary artery calcification in type 2 diabetes mellitus. *European heart journal* 2009; **30:** 3064-73.
29 Henry RM, Kostense PJ, Spijkerman AM*, et al.* Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. *Circulation* 2003; **107:** 2089-95.
30 Mitchell GF, Hwang SJ, Vasan RS*, et al.* Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation* 2010; **121:** 505-11.
31 Willum-Hansen T, Staessen JA, Torp-Pedersen C, Rasmussen S, Thijs L, Ibsen H, Jeppesen J. Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation* 2006; **113:** 664-70.

Table 1. CVD vs non-CVD in individuals with manifest T2D: univariate comparisons.

	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	ns
Medication					
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
Blood pressure					
SBP, mmHg	136 (123 - 150)	451	135 (124 - 147)	524	ns
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
Metabolic factors					
HbA1c, mmol/mol	56 (49.73 - 66)	443	55 (48 - 63)	517	0.025
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	ns
Renal function					
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
Vascular function					
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
Surrogate atherosclerosis markers					
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
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. ns, not significant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate; ABPI, Ankle Brachial Pressure Index; RHI, Reactive Hyperemia Index; PWV, Pulse Wave Velocity; CCA, common carotid artery; IMT, Intima Media Thickness.

Table 2. Logistic regression analysis of CVD status in individuals with manifest T2D, showing selected factors of interest.

	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)	ns
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)	ns
PWV, m/s	1.01 (0.95 - 1.08)	0.75	1.00 (0.92 - 1.08)	ns
Common carotid IMT Right, mm	2.59 (1.28 - 5.24)	8.0e-03	2.21 (1.00 - 4.88)	0.049
Common carotid IMT Left, mm	1.16 (0.63 - 2.16)	0.63	1.01 (0.51 - 2.03)	ns
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)	ns
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). ns, not significant; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ACR, ABPI, Ankle Brachial Pressure Index,; RHI, Reactive Hyperemia Index; PWV, Pulse Wave Velocity; IMT; CCA, common carotid artery; Intima Media Thickness; OR, odds ratio; CI, confidence interval.

Table 3. T2D vs Non-T2D irrespective of CVD status: univariate comparisons

Phenotype	**T2D with or without CVD** median (IQR)	**N**	**Non-T2D with or without CVD** median (IQR)	**N**	**p-value**
Male sex, n(%)	655 (66)	985	324 (63)	515	ns
Age, years	68 (63 - 74)	984	68 (62 - 74)	514	ns
BMI, kg/m^2	29.9 (26.8 - 33.5)	983	26.8 (24.5 - 29.4)	512	3.2e-31
Medication					
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
Blood pressure					
SBP, mmHg	135 (124 - 148)	975	130 (121 - 144)	509	2.8e-05
DBP, mmHg	76 (69.5 - 83)	976	76 (70 - 82)	510	ns
Pulse pressure, mmHg	58 (50 – 68)	975	54 (46 – 64)	513	1.1e-08
Metabolic factors					
HbA1c, mmol/mol	55.19 (48.63 - 65)	960	39 (37 - 42)	506	4.4e-173
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
Renal function					
Creatinine, serum, μmol/l	81.0 (69.0 - 94.8)	956	80.4 (70.0 - 91.1)	503	ns
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	ns
Vascular function					
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
Surrogate atherosclerosis markers					
ABPI Right	1.14 (1.04 - 1.22)	941	1.14 (1.06 - 1.22)	497	ns
ABPI Left	1.13 (1.03 - 1.22)	934	1.14 (1.05 - 1.21)	498	ns
Common carotid IMT Left, mm	0.91 (0.78 - 1.05)	896	0.86 (0.74 - 1.03)	475	1.9e-04
Common carotid IMT Right, mm	0.89 (0.77 - 1.03)	903	0.84 (0.74 - 0.98)	475	6.6e-05
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. ns, not significant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate; ABPI, Ankle Brachial Pressure Index; RHI, Reactive Hyperemia Index; PWV, Pulse Wave Velocity; IMT, Intima Media Thickness.

Table 4. 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	1.01 (1.01 - 1.02)	1.7e-04	1.00 (0.99 - 1.02)	ns
DBP	1.00 (0.99 - 1.01)	ns	0.99 (0.96 - 1.01)	ns
ABPI Right	1.00 (0.50 - 1.98)	ns	1.52 (0.40 - 5.78)	ns
ABPI Left	0.76 (0.38 - 1.53)	ns	1.00 (0.24 - 4.17)	ns
RHI	0.62 (0.51 - 0.76)	1.8e-06	0.94 (0.66 - 1.32)	ns
PWV	1.25 (1.18 - 1.33)	8.6e-13	1.07 (0.95 - 1.20)	ns
CCA IMT Right	2.29 (1.26 - 4.14)	6.5e-03	1.27 (0.45 - 3.58)	ns
CCA IMT Left	1.79 (1.05 - 3.06)	0.033	1.23 (0.51 - 2.98)	ns
Bulb Right	1.28 (0.98 - 1.67)	ns	1.01 (0.64 - 1.60)	ns
Bulb Left	1.32 (0.94 - 1.85)	ns	1.10 (0.618 - 1.96)	ns
eGFR	0.995 (0.99 – 1.00)	ns	1.02 (0.99 – 1.05)	ns

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). sns, not significant; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ACR, ABPI, Ankle Brachial Pressure Index,; RHI, Reactive Hyperemia Index; PWV, Pulse Wave Velocity; IMT, Intima Media Thickness; OR, odds ratio; CI, confidence interval.

Table 5. Spearman Rho correlations between vascular changes in all participants

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

ns, not significant; ABPI, Ankle Brachial Index; CCA, common carotid artery; IMT, Intima Media Thickness; PWV, Pulse Wave Velocity; RHI, Reactive Hyperemia Index

Supplemental data

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(%)	179 (71.3)	251	145 (54.9)	264	1.2e-4
Age, years	70 (64 - 75)	251	66 (59 - 71)	266	3.9e-7
BMI, kg/m^2	27.4 (25.0 - 30.2)	249	26.2 (24.1 - 28.8)	267	9.0e-4
Medication					
Statin use, n(%)	216 (86.1)	251	52 (20.0)	260	1.6e-50
Anti-hypertensive treatment use, n(%)	220 (87.7)	251	81 (30.9)	262	6.8e-39
Blood pressure					
SBP, mmHg	130 (123 - 144)	247	131 (120 - 144)	266	ns
DBP, mmHg	75 (69 - 81)	248	77 (72 - 84)	266	0.0026
Pulse pressure, mmHg	56 (48 – 65)	247	52 (45 – 62)	263	0.0011
Metabolic factors					
HbA1c, mmol/mol	40 (37.4 – 42.0)	248	39 (36.0 – 41.0)	258	8.4e-5
Tot Cholesterol, mmol/l	4.07 (3.58 – 4.70)	245	5.25 (4.70 – 5.98)	259	3.8e-34
HDL, mmol/l	1.30 (1.06 - 1.60)	243	1.50 (1.25 - 1.84)	257	4.4e-8
LDL, mmol/l	2.20 (1.81 - 2.70)	236	3.26 (2.70 - 3.80)	248	7.8e-30
Triglycerides, mmol/l	1.16 (0.89 - 1.61)	244	1.20 (0.88 - 1.61)	256	ns
Renal function					
Creatinin, serum, μmol/l	83 (73 - 95)	245	77 (67 - 87)	248	6.1e-6
ACR, mg/mmol	0.70 (0.43 - 1.50)	182	0.60 (0.40 – 1.00)	220	0.011
eGFR, mL/min per 1.73 m2	60.5 (51.6 - 77.5)	245	74.4 (58.7 - 86.2)	258	2.8e-8
Vascular function					

RHI	2.23 (1.77 - 2.65)	235	2.36 (1.98 - 2.81)	259	0.0020
PWV, m/s	10.1 (8.44 - 11.80)	208	9.4 (8.17 - 10.80)	244	0.0011
Pressure index and ultrasound					
ABPI Right	1.13 (1.03 - 1.22)	241	1.15 (1.08 - 1.23)	260	0.033
ABPI Left	1.13 (1.03 - 1.21)	241	1.14 (1.07 - 1.21)	261	ns
Common carotid IMT Right, mm	0.87 (0.77 - 1.01)	227	0.81 (0.73 - 0.95)	248	0.0023
Common carotid IMT Left, mm	0.88 (0.76 - 1.04)	226	0.85 (0.71 - 1.00)	249	7.3e-3
Carotid bulb Right, mm	1.09 (0.92 - 1.42)	196	0.98 (0.85 - 1.18)	234	1.6e-5
Carotid bulb Left, mm	1.15 (0.97 - 1.40)	195	1.00 (0.84 - 1.24)	236	1.4e-6

Binary variables are reported as n(%). Differences between groups were tested by chi2 or Mann–Whitney U tests, as appropriate. ns, not significant; BMI, body mass index;SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate; ABPI, Ankle Brachial Pressure Index; RHI, Reactive Hyperemia Index; PWV, Pulse Wave Velocity; IMT, Intima Media Thickness.

Supplemental table 2. Backward linear regressions in T2D subjects only, showing covariates with $p<0.05$

Dependent variable	**Independent variable**	**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.
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 center variables. Continuous variables were standardized to mean=0 and std=1.

Supplemental table 3. Spearman Rho correlations between CVD risk factors and vascular changes in all T2D individuals

Variable		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	ns	ns	3.50E-18	1.25E-14	5.09E-18	1.47E-10	4.55E-22	ns
BMI	rho	-0.031	-0.027	-0.057	-0.117	-0.039	-0.056	0.114	-0.118
	p	ns	ns	ns	9.44E-04	ns	ns	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	ns	ns	1.25E-05	0.002	ns	ns	3.80E-10	ns
HbA1c	rho	-0.039	-0.018	0.057	0.059	0.019	0.053	0.122	-0.06
	p	ns	ns	ns	ns	ns	ns	7.54E-04	ns
Tot Chol	rho	-0.026	-0.026	-0.079	-0.054	0.02	0.007	-0.071	0.065
	p	ns	ns	0.028	ns	ns	ns	ns	ns
LDL	rho	0.016	0.015	-0.052	-0.039	0.04	0.038	-0.052	0.057
	p	ns	ns	ns	ns	ns	ns	ns	ns
HDL	rho	0.041	0.044	-0.052	-0.055	-0.093	-0.106	-0.098	0.115
	p	ns	ns	ns	ns	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	ns	ns	ns	ns	ns	0.003
Creatinine	rho	-0.004	-0.036	0.168	0.147	0.131	0.117	0.161	-0.043
	p	ns	ns	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	ns	ns	3.13E-09	3.88E-07	2.16E-07	7.44E-05	6.11E-09	ns
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	ns	1.90E-10	ns
T2D duration	rho	-0.053	-0.062	0.185	0.164	0.060	0.028	0.186	-0.047
	p	ns	ns	2.30e-7	4.40e-6	ns	ns	2.27e-7	ns

ns, not significant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate; ABPI, Ankle Brachial Pressure Index; RHI, Reactive Hyperemia Index; PWV, Pulse Wave Velocity; IMT, Intima Media Thickness.

Supplemental table 4. Spearman Rho correlations between CVD risk factors and vascular changes 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	ns	ns	1.49E-20	2.79E-15	4.59E-26	1.06E-11	2.60E-41	ns
BMI	rho	0.041	0.037	0.094	0.015	0.1	0.117	0.193	-0.263
	p	ns	ns	ns	ns	0.030	0.011	3.56E-05	3.67E-09
SBP	rho	-0.083	-0.098	0.274	0.217	0.338	0.303	0.457	0.106
	p	ns	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	ns	ns	ns	ns	ns	ns	ns	ns
HbA1c	rho	-0.072	-0.131	0.142	0.13	0.121	0.088	0.12	-0.059
	p	ns	0.004	0.004	0.007	0.009	ns	0.012	ns
Tot Chol	rho	-0.046	0.024	-0.114	-0.152	-0.074	-0.032	-0.074	0.171
	p	ns	ns	0.019	0.002	ns	ns	ns	1.71E-04
LDL	rho	-0.002	0.059	-0.055	-0.1	-0.033	0.017	-0.055	0.097
	p	ns	ns	ns	0.044	ns	ns	ns	0.037
HDL	rho	-0.066	-0.02	-0.115	-0.123	-0.123	-0.138	-0.135	0.238
	p	ns	ns	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	ns	ns	ns	ns	ns	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	3.77	0.00

								E-04	2
eGFR	rho	-0.097	-0.074	-0.217	-0.199	-0.266	-0.179	-0.289	-0.113
	p	0.033	ns	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	ns	ns	0.007	9.46E-06	0.006	0.001	ns

ns, not significant; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACR, albumin creatinine ratio; eGFR, estimated glomerular filtration rate; ABPI, Ankle Brachial Pressure Index; RHI, Reactive Hyperemia Index; PWV, Pulse Wave Velocity; IMT, Intima Media Thickness