

# High burden of coronary atherosclerosis in patients with a new diagnosis of type 2 diabetes

Diabetes & Vascular Disease Research  
2017, Vol. 14(6) 468–476  
© The Author(s) 2017  
Reprints and permissions:  
sagepub.co.uk/journalsPermissions.nav  
DOI: 10.1177/1479164117728014  
journals.sagepub.com/home/dvr  


Monija Mrgan<sup>1</sup>, Kristian L Funck<sup>2</sup>, Sara Gaur<sup>3</sup>,  
Kristian A Øvrehus<sup>4</sup>, Damini Dey<sup>5</sup>, Martin W Kusk<sup>6</sup>,  
Bjarne L Nørgaard<sup>3</sup>, Jørgen B Gram<sup>7,8</sup>, Michael H Olsen<sup>9,10</sup>,  
Jeppe Gram<sup>11</sup> and Niels Peter R Sand<sup>1,12</sup>

## Abstract

**Purpose:** The purposes of this study were to compare the presence, extent and composition of coronary plaques in asymptomatic patients with newly diagnosed type 2 diabetes to age- and sex-matched controls.

**Methods:** Patients with newly diagnosed (<1 year) type 2 diabetes ( $n=44$ ) and controls ( $n=44$ ) underwent contrast-enhanced coronary computed tomography angiography. Advanced plaque analysis including total plaque volume and volumes of plaque components (calcified plaque and non-calcified plaque, including low-attenuation [low-density non-calcified plaque]) was performed using validated semi-automated software.

**Results:** Coronary artery calcification was more often seen in patients with type 2 diabetes (66%) versus controls (48%),  $p<0.05$ . Both the absolute volume (median; interquartile range) of low-density non-calcified plaque ( $7.9\text{mm}^3$ ;  $0\text{--}50.5\text{mm}^3$  vs  $0$ ;  $0\text{--}34.3\text{mm}^3$ ,  $p<0.05$ ) and the increase in low-density non-calcified plaque ratio in relation to total plaque volume ( $\tau=0.5$ ,  $p<0.001$ ) were significantly higher in patients with type 2 diabetes. More patients with type 2 diabetes had spotty calcification (31% vs 0%,  $p<0.05$ ). By multivariate analysis, the presence of any low-density non-calcified plaque was higher in males (odds ratio: 4.06,  $p<0.05$ ), who also demonstrated a larger low-density non-calcified plaque volume ( $p<0.001$ ). The presence and extent of low-density non-calcified plaque increased with age, smoking, hypertension and hyperglycaemia, all  $p<0.05$ .

**Conclusion:** Asymptomatic patients with newly diagnosed type 2 diabetes had plaque features associated with increased vulnerability as compared with age- and sex-matched controls.

## Keywords

Coronary plaque, coronary computed tomography, low-density plaque, type 2 diabetes

<sup>1</sup>Department of Cardiology, Hospital of Southwest Denmark, Esbjerg, Denmark

<sup>2</sup>Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark

<sup>3</sup>Department of Cardiology, Aarhus University Hospital, Aarhus, Denmark

<sup>4</sup>Department of Cardiology, Odense University Hospital, Odense, Denmark

<sup>5</sup>Biomedical Imaging Research Institute, Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA, USA

<sup>6</sup>Department of Radiology, Hospital of Southwest Denmark, Esbjerg, Denmark

<sup>7</sup>Unit for Thrombosis Research, University of Southern Denmark, Esbjerg, Denmark

<sup>8</sup>Department of Clinical Biochemistry, Hospital of Southwest Denmark, Esbjerg, Denmark

<sup>9</sup>Cardiology Section, Department of Internal Medicine, Holbæk Hospital, Holbæk, Denmark

<sup>10</sup>Centre for Individualized Medicine in Arterial Diseases, Odense University Hospital, University of Southern Denmark, Odense, Denmark

<sup>11</sup>Department of Endocrinology, Hospital of Southwest Denmark, Esbjerg, Denmark

<sup>12</sup>Department of Regional Health Research, University of Southern Denmark, Odense, Denmark

## Corresponding author:

Monija Mrgan, Department of Cardiology, Hospital of Southwest Denmark, Finsensgade 35, 6700 Esbjerg, Denmark.  
Email: momrg@hotmail.com

## Introduction

Coronary artery disease is a major cause of morbidity and mortality in the industrial world.<sup>1,2</sup> Several studies have shown a relationship between type 2 diabetes (T2D), an increased risk of coronary artery disease and a poor long-term prognosis,<sup>3,4</sup> findings that have been strengthened by studies demonstrating advanced cardiovascular involvement even in asymptomatic patients with T2D.<sup>5,6</sup> However, the available information on coronary artery disease in patients with T2D relies on studies of diabetes populations with either long diabetes duration<sup>5,7</sup> or known cardiovascular disease.<sup>8,9</sup> The literature regarding coronary artery disease and coronary plaque morphology in patients with newly diagnosed T2D is sparse.<sup>10,11</sup>

Contrast-enhanced coronary computed tomography angiography (CCTA) has evolved as a robust imaging modality for ruling out significant coronary artery disease.<sup>12,13</sup> Moreover, CCTA permits detailed non-invasive coronary plaque assessment.<sup>14,15</sup>

The purposes of this study were to evaluate the presence, extent and composition of coronary plaques in asymptomatic patients with newly diagnosed (<1 year) T2D and to compare these findings with age- and sex-matched controls.

## Methods

### Patients with T2D

From March 2014 to August 2016, a total of 213 adult patients diagnosed with T2D (haemoglobin A1c > 47 mmol/mol and plasma glucose > 7 mmol/L) within the last year were identified via general practitioners or outpatient clinics in the Region of Southern Denmark and invited by letter to participate in the study. A total of 115 (54%) patients agreed to participate. Patients were systematically evaluated by questionnaires and examination for symptoms of or known cardiovascular disease (defined as previous myocardial infarction, angina pectoris, stroke/transient ischaemic attack and/or peripheral atherosclerosis), in which case they were excluded from the study. Two patients with cardiovascular disease were excluded. Patients with pregnancy or inability to provide informed consent were also excluded. Patients with the following characteristics were excluded from the study: renal insufficiency (estimated glomerular filtration rate < 45 mL/min or serum creatinine > 140 micromole/L), body mass index (BMI) > 35 kg/m<sup>2</sup>, absence of sinus rhythm, contraindications to iodinated contrast agents and inability to perform a 10-s breath hold.<sup>16</sup>

### Control group

In total, 72 controls were recruited from the general population by advertisement in the local press. Controls were systematically evaluated by questionnaires and

examination for cardiovascular disease (criteria consistent with those described for patients with T2D) and T2D (undiagnosed T2D in this group was excluded by fasting plasma glucose and by an oral glucose tolerance test) were excluded. Seven controls with cardiovascular disease were excluded. In addition, exclusion criteria were consistent with those described for patients with T2D. Only 52 of controls met the inclusion criteria and underwent CCTA.

### Matching

Patients with T2D were matched 1:1 by age ( $\pm$ <1 year) and sex, giving a total of 44 persons in each group. Figure 1 summarizes the selection of study populations.

### Ethical considerations

The scientific protocols were approved by the Regional Scientific Ethical Committee for Southern Denmark (S-20140137 and S-1-10-72-349-13) and by the Danish Data Protection Agency (2008-58-0035 and 1-16-02-505-13) and were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all individual participants included in the study. The study is registered at ClinicalTrials.gov ID: NCT03022344 and protocol ID: 44110.

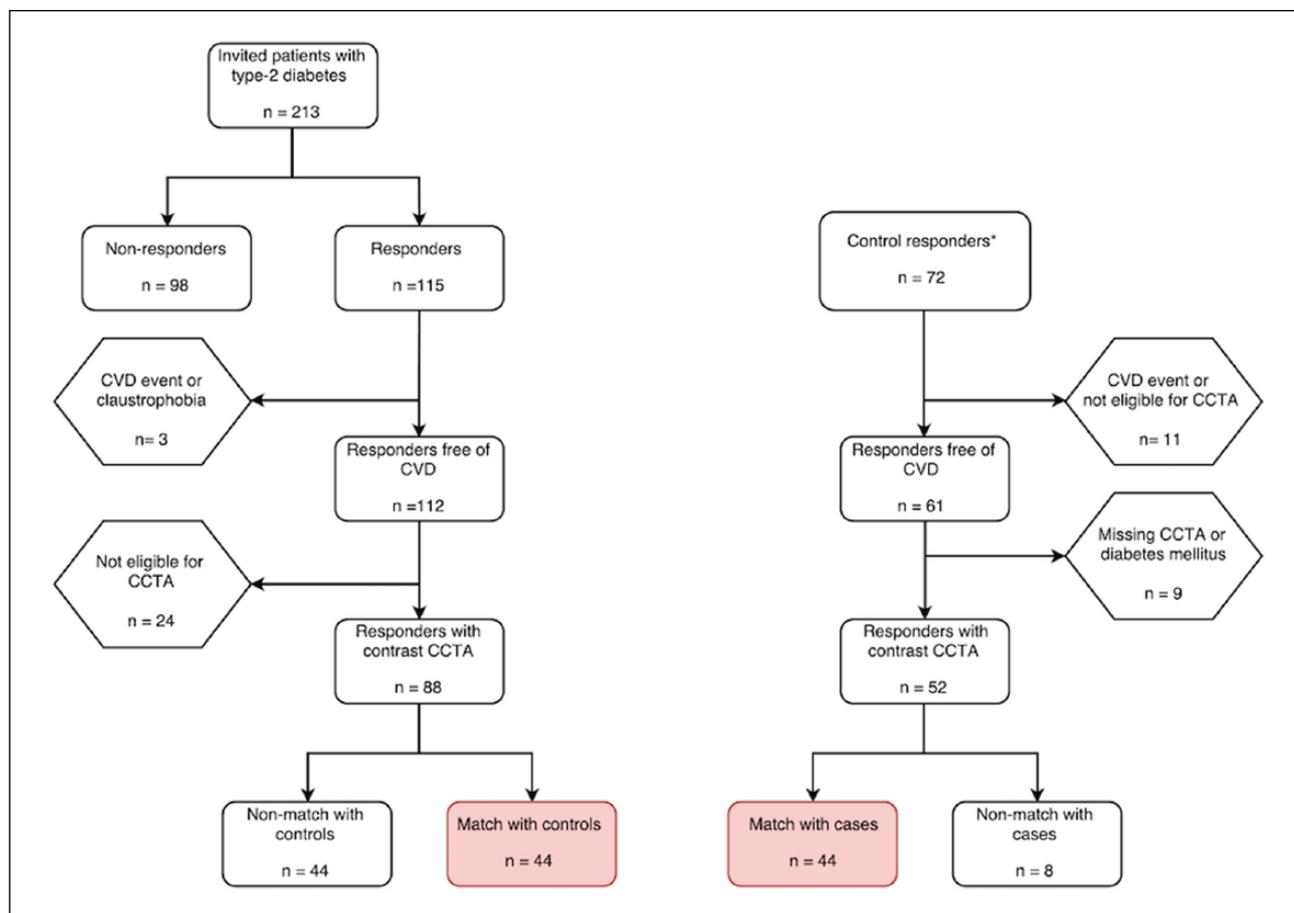
### Demographic data

Medical history, current medications and smoking habits were obtained from questionnaires. Height, weight and waist-hip ratio were measured. Venous blood samples were taken under fasting conditions and were analysed for low-density lipoprotein, high-density lipoprotein, triglycerides, total cholesterol, haemoglobin A1c, fasting glucose, C-reactive protein and creatinine. Glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.<sup>17</sup>

Morning midstream urine samples were examined for urine albumin/creatinine ratio. Brachial blood pressures were measured by oscillometric techniques over 24 h, using TM-2430 (A&D Co., Ltd, Saitama, Japan).<sup>18</sup> Blood pressures were defined as mean values of the 24-h recordings. Hypertension was defined as blood pressures  $\geq$ 140/90 mm Hg or treatment with at least one antihypertensive agent. Hypercholesterolaemia was defined as total cholesterol  $\geq$ 5 mmol/L or low-density lipoprotein  $\geq$ 3 mmol/L or treatment with at least one lipid-lowering agent. Abdominal obesity was defined as a waist-hip ratio above 0.90 for males and above 0.85 for females, or a BMI above 30.0 kg/m<sup>2</sup>.

### Cardiac computed tomography

**Acquisition.** Image acquisition was performed in accordance with guidelines defined by the Society of



**Figure 1.** Flow diagram of the study populations (for further information, see text).

Cardiovascular Computed Tomography.<sup>19</sup> Both patients with T2D and controls underwent CCTA (Siemens SOMATOM Definition Flash, Forchheim, Germany). Pre-scan oral and/or intravenous beta-receptor blockers were administered, targeting a heart rate  $\leq 60$  beats/min. Sublingual nitroglycerine, dose of 0.5 mg, was administered immediately before the scanning. Contrast-enhanced CCTA (Optiray™ 350 mgI/mL [Mallinckrodt]) was undertaken using the following settings: gantry rotation time 280 ms, 2 mm  $\times$  64 mm  $\times$  0.6 mm collimation, 100 or 120-kV tube voltage (depending on body geometry). A reference mAs of 320 with automatic tube current modulation was employed. Scans were prospective step and shoot, gated 65%–75% of the R-R interval. Images were reconstructed at 65%, 70% and 75% of the R-R interval, using iterative reconstruction (SAFIRE level 3) and kernel B31 (medium–smooth). Slice thickness was 0.6 mm with 0.3-mm interval. Both patients with T2D and controls underwent a standard non-contrast scan, with a reference mAs of 50 for assessment of coronary artery calcifications (CACs) by the Agatston score.<sup>16</sup>

**Data analyses.** Reconstructed CCTA images were transferred to a workstation, syngo.via (Siemens Healthcare)

for post processing. One experienced computed tomography (CT)-cardiologist, blinded to the clinical data, analysed all CCTA data.

Images were deemed non-evaluable if artefacts affected  $\geq 3$  coronary segments. A modified 17-segment model was used for allocation of plaques to a distinct vessel territory.<sup>20</sup> All coronary plaques were evaluated by visual assessment according to the following indicative criteria for coronary artery disease: non-obstructive (1%–29% stenosis), borderline (30%–69% stenosis), obstructive (70%–99% stenosis) and occlusive (100%) plaque. Images of the best phase of the cardiac cycle for visualization of coronary arteries were transferred to a research workstation for plaque assessment. CAC were quantified by non-contrast CCTA and were assessed by summing up the scores from all foci in the coronary arteries, expressed in Agatston units (U), using dedicated software (syngo Calcium Scoring, Siemens Healthcare).

**Plaque quantification.** Coronary segments  $> 2$  mm with plaques (tissue structures  $> 1 \text{ mm}^2$ )<sup>21,22</sup> were analysed by one trained reader using a validated semi-automated software (AutoPlaque, version 2.0, Cedars-Sinai Medical Center, Los Angeles, CA, USA).<sup>14,15,21</sup> CCTA images were

examined in multiplanar reformats, and a circular region of interest (normal reference blood pool) was placed in the aorta. Scan-specific thresholds for non-calcified plaque (NCP) and calcified plaque (CP) volumes were automatically generated.<sup>15</sup> Adjustments were made if necessary. Coronary plaque burden was defined as (plaque volume/vessel volume)  $\times$  100%. Remodelling index was calculated as maximum lesion vessel area/area of a proximal normal reference point.<sup>23</sup> Low-density non-calcified plaque (LD-NCP) was defined as a plaque with components attenuation  $<30$  Hounsfield units.<sup>23</sup> The part of the NCP that was not classified as LD-NCP was defined as the non-low-density non-calcified plaque (non-LD-NCP). All coronary plaques were categorized as belonging to one of the three vessel territories. The left main was considered part of either left anterior descending artery or left circumflex artery.<sup>22</sup> The analyses were performed by one experienced reader, who was blinded to all other patient data.

**Intra-observer variability.** The intra-observer variation for the assessment of plaque components were evaluated in 16 patients (8 patients with T2D and 8 controls) with an Agatston score  $>0$ . Studies were re-analysed 3–4 weeks after the initial evaluation by the same reader.

## Statistical analysis

The design of the study was a matched case-control study in which each patient with T2D was matched to a control by age ( $\pm <1$  year) and sex. Sample size estimations were performed for comparison of LD-NCP between patients with T2D and controls. From previous studies, the standard deviation (SD) on LD-NCP was known to be approximately  $25 \text{ mm}^3$ .<sup>22</sup> With a beta=0.2 and an alpha=0.05, it was calculated that 44 patients in each group were needed to obtain the required strength of the study.

Shapiro–Wilk test was used for testing the normal distribution of data. Continuous variables were presented as mean values and 95% confidence interval if normally distributed or median and interquartile ranges (IQRs) if non-normally distributed. Differences in baseline demographics between patients with T2D and controls (group-wise differences) were determined using paired *t*-test for normally distributed variables and Wilcoxon signed-rank test for non-normally distributed variables. Categorical variables were presented by frequencies and corresponding percentages.

LD-NCP data were characterized by a point mass at zero followed by a highly skewed continuous distribution, that's why a two-part model was chosen for the regression analyses to evaluate the association with the independent variables. First, a logistic regression model was used to determine the association between covariates and the presence or absence of LD-NCP. Second, the association between covariates and the extent of LD-NCP volumes among participants with LD-NCP was determined by a

generalized linear model with the gamma distribution using link-name identity. In the model, the following covariates were included: age, sex, BMI, hypertension, hypercholesterolaemia, smoking (ever smoker vs non-smokers) and haemoglobin A1c.

Differences in proportions between matched pairs were analysed by McNemar's test. Kruskal–Wallis test and Kendall's rank correlation were used to evaluate associations between plaque components and total plaque volume tertiles. Intra-observer variability of plaque characteristics was analysed using the paired *t*-test.

A *p*-value  $<0.05$  was considered statistically significant. All statistical analyses were performed using Stata software, version 14.0 (StataCorp LLC, College Station, TX, USA).

## Results

### Clinical characteristics

Clinical characteristics are presented in Table 1. Patients with newly diagnosed T2D were more often treated with 2 or more antihypertensive (30% vs 16%,  $p < 0.05$ ) or cholesterol-lowering (52% vs 18%,  $p < 0.05$ ) drugs compared to controls. Among patients with T2D, 6% received both oral medication and insulin therapy, 76% were treated with oral medication only and 18% had diet-controlled diabetes. Additionally, patients with newly diagnosed T2D had lower low-density lipoprotein, high-density lipoprotein and total cholesterol. Mean haemoglobin A1c was  $45 \text{ mmol/mol}$ , which is within the normal range ( $<47 \text{ mmol/mol}$ ).

### Contrast-enhanced CCTA and plaque analysis

There was no difference in heart rate (median; IQR) during CCTA between patients with T2D and controls (60 beats/min; 54–63 beats/min vs 59 beats/min; 54–63 beats/min,  $p = 0.98$ ). Significantly higher radiation doses (median; IQR) were applied in patients with T2D (435 mGy cm; 308–497 mGy cm) versus controls (238 mGy cm; 210–289 mGy cm),  $p < 0.001$ . The numbers of patients receiving 120 keV were equal in patients with T2D ( $n = 37$ ) and controls ( $n = 36$ ),  $p = 0.32$ .

CAC was identified in 29 (66%) patients with T2D versus 21 (48%) in controls,  $p < 0.05$ , Table 2. The Agatston score was significantly higher in patients with T2D,  $p < 0.05$ . Overall, we registered 174 segments with plaques in 50 participants, of which 96 (56%) were found in patients with T2D. The prevalence of coronary obstruction ( $\geq 70\%$  stenosis) was 9.1% in patients with T2D versus 6.8% in controls,  $p = 0.50$ . No plaques were found in neither patients nor controls with an Agatston score of zero.

The assessment of coronary plaque composition is illustrated in Table 2. The LD-NCP volume was significantly higher in patients with T2D compared to controls,

**Table 1.** Clinical characteristics of the study populations.

	Type 2 diabetes, <i>n</i> =44	Controls, <i>n</i> =44	<i>p</i> -value
<b>Demographics</b>			
Age, years <sup>a</sup>	60.2 (9.3)	60.4 (9.5)	
Male <sup>b</sup>	27 (61)	27 (61)	
Ethnicity (Caucasian) <sup>b</sup>	44 (100)	44 (100)	1.000
<b>Risk factors</b>			
Body mass index (kg/m <sup>2</sup> ) <sup>a</sup>	29.6 (3.8)	26.7 (2.8)	<0.001
Waist-hip ratio <sup>a</sup>	1.0 (0.1)	0.9 (0.1)	<0.001
Abdominal obesity <sup>b</sup>	41 (93)	30 (68)	0.003
Ever smoker <sup>b</sup>	28 (64)	20 (46)	0.073
Hypertension <sup>b</sup>	30 (68)	14 (32)	0.002
Hypercholesterolaemia <sup>b</sup>	35 (80)	37 (84)	0.563
<b>Medical therapy</b>			
Cholesterol-lowering medication <sup>b</sup>	23 (52)	8 (18)	0.002
Antihypertensive medication <sup>b</sup>	23 (52)	13 (30)	0.025
<b>24-h blood pressure</b>			
Systolic blood pressure <sup>a</sup>	129.9 (11.7)	123.1 (9.3)	0.015
Diastolic blood pressure <sup>a</sup>	74.6 (6.4)	75.7 (7.5)	0.442
Pulse rate, beats per minute <sup>a</sup>	71.9 (9.6)	70.6 (8.5)	0.479
<b>Laboratory findings</b>			
Fasting glucose, mmol/L	7.4 (7.1–8.1)	5.6 (5.4–6.0)	<0.001
Haemoglobin A1c, mmol/mol <sup>c</sup>	45 (43.0–49.5)	38 (36.0–40.0)	<0.001
Total-cholesterol, mmol/L <sup>a</sup>	4.7 (1.1)	5.4 (1.1)	0.005
Low-density lipoprotein, mmol/L <sup>a</sup>	2.7 (0.9)	3.2 (1.0)	0.014
High-density lipoprotein, mmol/L <sup>a</sup>	1.2 (0.3)	1.6 (0.5)	<0.001
Triglycerides, mmol/L <sup>c</sup>	1.7 (1.3–2.7)	0.9 (0.7–1.3)	<0.001
eGFR, mL/min <sup>a</sup>	83.6 (16.1)	79.2 (9.0)	0.071
Serum creatinine, µmol/L <sup>a</sup>	76.3 (12.9)	77.5 (14.4)	0.583
Urine albumin creatinine ratio, mg/g <sup>c</sup>	6.5 (5.0–11.0)	0.9 (0.0–2.8)	<0.001
C-reactive protein, mg/L <sup>c</sup>	1.6 (0.8–3.0)	1.1 (0.3–2.7)	0.404

eGFR: estimated glomerular filtration rate.

<sup>a</sup>Values are mean (SD).

<sup>b</sup>Values are *n* (%).

<sup>c</sup>Values are median (IQR).

whereas total plaque volume, CP and NCP volumes were non-significantly higher in patients with T2D. Furthermore, the LD-NCP plaque burden was significantly higher in patients with T2D compared to controls, and more patients with T2D versus controls had spotty calcification (31% vs 0%,  $p < 0.05$ ), while the remodelling index (median; IQR) was similar in the two groups (1.5; 1.5–2.0 vs 1.5; 1.4–1.6,  $p = 0.16$ ). The association between the ratio of plaque components and total plaque volume tertiles in patients with T2D and controls is shown in Figure 2. The LD-NCP ratio increased with higher total plaque volume in patients with T2D ( $\tau = 0.5$ ,  $p < 0.005$ ) but not in controls. There were no differences in BMI and radiation doses between tertiles of plaque volumes, neither in patients with T2D nor in controls.

Multivariate analyses for the entire population are presented in Table 3. Men had a higher risk for the presence of any LD-NCP (odds ratio [OR]: 4.06,  $p < 0.05$ ) and demonstrated a larger LD-NCP volume than women ( $p < 0.001$ ).

BMI was positively associated with the occurrence of any LD-NCP, while the inverse correlation was found between BMI and LD-NCP volumes. The presence and extent of LD-NCP increased with age, smoking, hypertension and the degree of hyperglycaemia.

A good intra-observer agreement on all of the applied plaque analysis components was found. No significant differences in mean NCP (mean difference: 7.4 mm<sup>3</sup>,  $p = 0.88$ ), CP (mean difference: 0.76 mm<sup>3</sup>,  $p = 0.91$ ) or LD-NCP volumes (mean difference: 0.22 mm<sup>3</sup>,  $p = 0.85$ ) were observed.

## Discussion

In this study, we evaluated the differences in the presence, extent and composition of coronary plaques between asymptomatic patients with newly diagnosed T2D and sex- and age-matched controls. The major finding of this study is related to the differences in the LD-NCP component between patients with T2D and controls both in

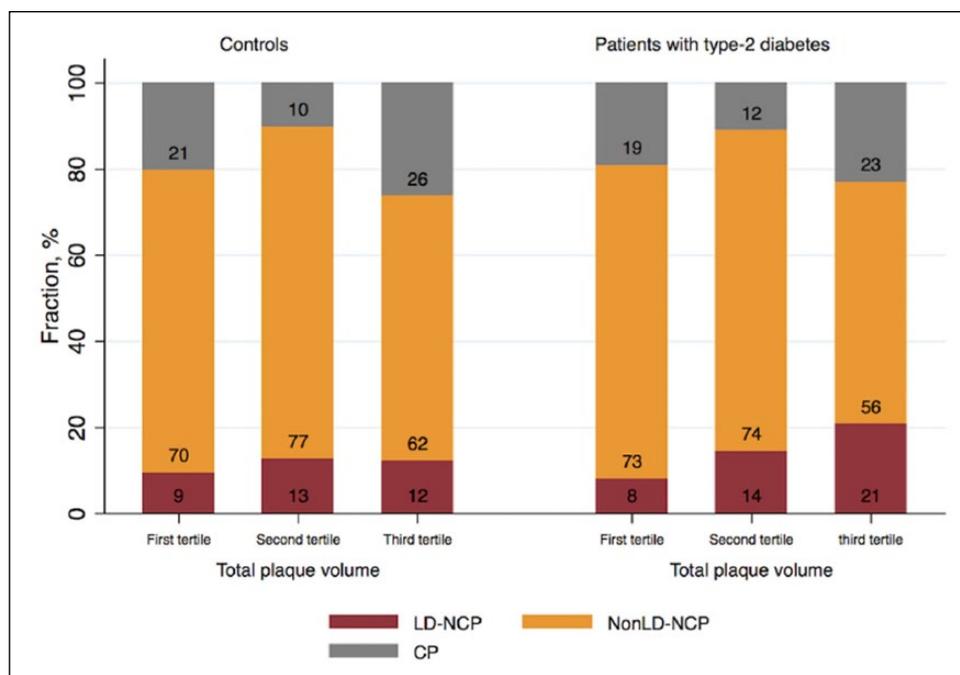
**Table 2.** Assessment of coronary artery disease and plaque composition.

	Type 2 diabetes, n = 44	Controls, n = 44	p-value
Number of affected coronary vessels			
0 vessel <sup>a</sup>	15 (34)	23 (52)	0.037
1 vessel <sup>a</sup>	8 (18)	5 (11)	
2 vessels <sup>a</sup>	6 (14)	5 (11)	
3 vessels <sup>a</sup>	15 (34)	11 (25)	
Agatston score, U <sup>a</sup>	29 (66)	21 (48)	0.021
Agatston score			
Score 0, U <sup>a</sup>	15 (34)	23 (52)	0.020
Score 1–99, U <sup>a</sup>	14 (32)	13 (29)	
Score 100–399, U <sup>a</sup>	7 (16)	4 (9)	
Score >400, U <sup>a</sup>	8 (18)	4 (9)	
Plaque composition			
Absolute plaque volumes, mm <sup>3</sup>			
Total plaque volume <sup>b</sup>	70.6 (0–437.8)	0 (0–331.5)	0.052
CP volume <sup>b</sup>	4.9 (0–72.0)	0 (0–26.2)	0.069
NCP volume <sup>b</sup>	57.9 (0–394.3)	0 (0–300.9)	0.085
Non-LD-NCP volume <sup>b</sup>	49.5 (0–297.5)	0 (0–249.3)	0.183
LD-NCP volume <sup>b</sup>	7.9 (0–50.5)	0 (0–34.3)	0.025
Plaque burden, %			
Total plaque burden <sup>b</sup>	1.7 (0–13.0)	0 (0–10.5)	0.122
CP burden <sup>b</sup>	1.7 (0–13.0)	0 (0–10.5)	0.122
NCP burden <sup>b</sup>	13.4 (0–114.5)	0 (0–87.9)	0.085
Non-LD-NCP burden <sup>b</sup>	11.6 (0–87.9)	0 (0–77.5)	0.268
LD-NCP burden <sup>b</sup>	1.6 (0–19.3)	0 (0–9.9)	0.012

Non-LD-NCP: non-low-density non-calcified plaque; LD-NCP: low-density non-calcified plaque; CP: calcified plaque; NCP: non-calcified plaque.

<sup>a</sup>Values are n (%).

<sup>b</sup>Values are median (IQR).

**Figure 2.** Ratio of coronary plaque components in relation to total plaque volume.

The absolute values of total plaque volume were divided into tertiles with the following values given as median (IQR): first tertile: 70 mm<sup>3</sup> (9–96), second tertile: 361 mm<sup>3</sup> (274–384) and third tertile: 776 mm<sup>3</sup> (665–988). CP: calcified plaque; LD-NCP: low-density non-calcified plaque; Non-LD-NCP: non-low-density non-calcified plaque.

**Table 3.** Association between LD-NCP and cardiac risk factors – multivariate analysis.

	Presence of LD-NCP, n = 88		Extent of LD-NCP, n = 88	
	Logistic regression		Generalized linear model	
	OR	p-value	Coefficient	p-value
Sex	4.06	0.022	44.3	<0.001
Age	4.48	<0.001	43.7	<0.001
Body mass index	3.00	0.008	-21.2	0.003
Ever smoker	3.00	0.073	10.3	0.004
Hypercholesterolaemia	0.91	0.947	8.9	0.586
Hypertension	1.22	0.731	33.8	<0.001
Haemoglobin A1c	1.11	0.727	10.4	0.004

LD-NCP: low-density non-calcified plaque; OR: odds ratio.  
The model is for both patients with T2D and controls.

absolute and relative terms. Our findings are consistent with previous reports that patients with T2DM have a higher atherosclerotic burden<sup>3,24,25</sup> compared to populations without T2D,<sup>9,26,27</sup> but this study extends previous observations by demonstrating that patients with T2D have a greater proportion of LD-NCP and more often spotty calcifications, indicating a vulnerable coronary plaque composition in patients with T2D already at the time of diagnosis. However, due to the limited number of patients in this observational study, it could not be proved which of the known cardiovascular risk factors contributed the most to the observed adverse coronary plaque structure in patients with newly diagnosed T2D.

In this study, we found that asymptomatic patients with a new diagnosis of T2D have a higher extent of CAC compared to controls. These findings are in accordance with the results of a study comparing CAC in healthy individuals and asymptomatic patients with T2D.<sup>28</sup> However, in the latter study, patients had longer diabetes duration than in the current.

Although well validated, it should be acknowledged that the Agatston score is a semi-quantitative metric and only represents the CP component. The ability of contrast-enhanced CCTA to discriminate between different plaque components has been validated by comparison to intravascular ultrasound.<sup>29</sup> An intravascular ultrasound-based study by Amano et al.<sup>30</sup> reported a more lipid-rich coronary plaque content in patients with acute coronary syndromes and abnormal glucose tolerance. These lipid-rich plaque contents might correspond to the findings of previous autopsy studies, in which the prevalence of necrotic cores and inflammatory cells in coronary plaques among patients with T2D was high.<sup>31,32</sup> Such plaques may be more vulnerable to rupture and as a consequence may be related to a higher cardiovascular event rate. Moreover, these lipid-rich portions of coronary plaques might be identical to parts of the NCP described by CCTA, especially the low-density NCP. Indeed, recent studies report that the LD-NCP component is an important determinant

of acute coronary syndromes<sup>33,34</sup> and that coronary lesions with high LD-NCP content more frequently are associated with lesion-specific ischaemia compared to lesions without these adverse characteristics.<sup>22</sup> A CCTA-based study by Nakanishi et al.<sup>35</sup> supplements these results, demonstrating that symptomatic patients with diabetes have progressively increasing NCP components compared to patients without diabetes. Importantly, they showed that larger NCP volumes at baseline are associated with progression in the LD-NCP volumes. In line with the study by Nakanishi et al.,<sup>35</sup> we found that the degree of hyperglycaemia was independently associated with LD-NCP volumes.

As the structure of plaques are complex, the accurate mechanism of coronary plaque progression have not yet been proved but may be dependent on plaque composition, cardiovascular risk profile and level of inflammatory activation.<sup>10,36–38</sup> Lehman et al.<sup>38</sup> found that the differences in the rate of progression are dependent on plaque composition and that this progression may be greater for NCP compared with CP.

Coronary plaque composition has primarily been evaluated in T2D populations with long duration of T2D<sup>5,7</sup> or in patients with T2D with symptoms of suspected or known coronary artery disease.<sup>8,9</sup> This study adds knowledge by indicating a more fragile coronary atherosclerotic plaque structure even in the early stages of T2D. This study is too small for evaluating any independent effects of the parameters in the metabolic syndrome on plaque vulnerability, but at the time of diagnosis, asymptomatic patients with T2D are likely to have more advanced stages of coronary plaques due to the often long and silent pre-diagnostic stages of this disease.

The findings in this exploratory study have to be confirmed in larger trials that might promote further insight into plaque morphology and thereby help designing more effective treatment strategies in patients with T2D. Asymptomatic patients with newly diagnosed T2D often have atypical or no symptoms at all, even in the presence

of disease. Knowing that asymptomatic patients with T2D may have more vulnerable plaque already at diagnosis, a more aggressive treatment algorithm or screening for coronary artery disease might be considered at an early stage.

### Limitations

The number of patients in this study was small. As only 54% of the invited patients with T2D agreed to participate, we cannot exclude some degree of bias. In patients with T2D, a significantly higher radiation dose was applied. It is well known<sup>39,40</sup> that the interaction of different tissue structures with X-ray beams at different energies can create artefacts such as blooming, which might disturb exact volume-measurements of the distinct plaque components. However, in the lower plaque volume tertile, we found no differences in plaque composition between patients with T2D and controls despite differences in radiation doses. In addition, significant differences in plaque composition between tertiles of plaque volumes were found in patients with T2D despite identical radiation doses. Therefore, the findings in this study unlikely were affected by artefacts relating to the applied radiation dose.<sup>39,40</sup> In addition, it should be emphasized that plaque analyses were performed using a scan-adaptive plaque threshold algorithm.

In conclusion, this study indicates that asymptomatic patients with newly diagnosed T2D have a high burden of coronary atherosclerosis and that coronary plaque composition in these patients displays features associated with an increased vulnerability. Several of the traditional risk factors for coronary artery disease were present already at the time of diagnosis of T2D, but the contribution of each of these factors for the observed adverse plaque composition in asymptomatic patients newly diagnosed T2D could not be further elucidated.

### Acknowledgements

M.M. designed the study, collected, analysed and interpreted the data and wrote the report. M.M. analysed all coronary computed tomography angiography (CCTA) using AutoPlaque and did Agatston investigation; K.L.F. collected the control group and edited the manuscript; S.G. and K.A.Ø. trained M.M. in AutoPlaque and edited the manuscript. D.D. developed the analysis tool and reviewed the manuscript. M.W.K. performed CCTA data and edited the manuscript; B.L.N. contributed to the discussion and reviewed the manuscript; J.B.G. and J.G. designed the study and edited the manuscript; M.H.O. conceived and designed the study and edited the manuscript; N.P.R.S. conceived and designed the study, collected, analysed data and contributed to the discussion and reviewed the manuscript.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

### Funding

The Danish Diabetes Academy supported by the Novo Nordisk Foundation; The Faculty of Health Sciences, University of Southern Denmark; Department of Regional Health Research, Center Southwest, Denmark; Edith and Vagn Hedegaard Jensens Foundation, Karola Jørgensens Foundation and Sydvestjysk Cardiologisk Forskningsfund. The funders had no role in the study design, data collection, data analysis and interpretation, writing of the report or the decision to submit the article for publication.

### References

1. Bentzon JF, Otsuka F, Virmani R, et al. Mechanisms of plaque formation and rupture. *Circ Res* 2014; 114: 1852–1866.
2. Libby P, Ridker PM and Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature* 2011; 473: 317–325.
3. Elkeles RS, Godsland IF, Feher MD, et al. Coronary calcium measurement improves prediction of cardiovascular events in asymptomatic patients with type 2 diabetes: the PREDICT study. *Eur Heart J* 2008; 29: 2244–2251.
4. Haffner SM, Lehto S, Ronnema T, et al. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; 339: 229–234.
5. Scholte AJ, Schuijf JD, Kharagjitsingh AV, et al. Prevalence of coronary artery disease and plaque morphology assessed by multi-slice computed tomography coronary angiography and calcium scoring in asymptomatic patients with type 2 diabetes. *Heart* 2008; 94: 290–295.
6. Hadamitzky M, Hein F, Meyer T, et al. Prognostic value of coronary computed tomographic angiography in diabetic patients without known coronary artery disease. *Diabetes Care* 2010; 33: 1358–1363.
7. Kwan AC, May HT, Cater G, et al. Coronary artery plaque volume and obesity in patients with diabetes: the factor-64 study. *Radiology* 2014; 272: 690–699.
8. Pundziute G, Schuijf JD, Jukema JW, et al. Noninvasive assessment of plaque characteristics with multislice computed tomography coronary angiography in symptomatic diabetic patients. *Diabetes Care* 2007; 30: 1113–1119.
9. Zeina AR, Odeh M, Rosenschein U, et al. Coronary artery disease among asymptomatic diabetic and nondiabetic patients undergoing coronary computed tomography angiography. *Coron Artery Dis* 2008; 19: 37–41.
10. Lim S, Choi SH, Kim KM, et al. The association of rate of weight gain during early adulthood with the prevalence of subclinical coronary artery disease in recently diagnosed type 2 diabetes: the MAXWEL-CAD study. *Diabetes Care* 2014; 37: 2491–2499.
11. Kataoka Y, Yasuda S, Miyamoto Y, et al. Effects of voglibose and nateglinide on glycemic status and coronary atherosclerosis in early-stage diabetic patients. *Circ J* 2012; 76: 712–720.
12. Budoff MJ, Dowe D, Jollis JG, et al. Diagnostic performance of 64-multidetector row coronary computed tomographic angiography for evaluation of coronary artery stenosis in individuals without known coronary artery disease: results

- from the prospective multicenter ACCURACY (Assessment by Coronary Computed Tomographic Angiography of Individuals Undergoing Invasive Coronary Angiography) trial. *J Am Coll Cardiol* 2008; 52: 1724–1732.
13. Abdulla J, Abildstrom SZ, Gotzsche O, et al. 64-multislice detector computed tomography coronary angiography as potential alternative to conventional coronary angiography: a systematic review and meta-analysis. *Eur Heart J* 2007; 28: 3042–3050.
  14. Dey D, Cheng VY, Slomka PJ, et al. Automated 3-dimensional quantification of noncalcified and calcified coronary plaque from coronary CT angiography. *J Cardiovasc Comput Tomogr* 2009; 3: 372–382.
  15. Dey D, Schepis T, Marwan M, et al. Automated three-dimensional quantification of noncalcified coronary plaque from coronary CT angiography: comparison with intravascular US. *Radiology* 2010; 257: 516–522.
  16. Agatston AS, Janowitz WR, Hildner FJ, et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol* 1990; 15: 827–832.
  17. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150: 604–612.
  18. Palatini P, Frigo G, Bertolo O, et al. Validation of the A&D TM-2430 device for ambulatory blood pressure monitoring and evaluation of performance according to subjects' characteristics. *Blood Press Monit* 1998; 3: 255–260.
  19. Abbara S, Blanke P, Maroules CD, et al. SCCT guidelines for the performance and acquisition of coronary computed tomographic angiography: a report of the society of Cardiovascular Computed Tomography Guidelines Committee: endorsed by the North American Society for Cardiovascular Imaging (NASCI). *J Cardiovasc Comput Tomogr* 2016; 10: 435–449.
  20. Leipsic J, Abbara S, Achenbach S, et al. SCCT guidelines for the interpretation and reporting of coronary CT angiography: a report of the Society of Cardiovascular Computed Tomography Guidelines Committee. *J Cardiovasc Comput Tomogr* 2014; 8: 342–358.
  21. Ovrehus KA, Schuhbaeck A, Marwan M, et al. Reproducibility of semi-automatic coronary plaque quantification in coronary CT angiography with sub-mSv radiation dose. *J Cardiovasc Comput Tomogr* 2016; 10: 114–120.
  22. Gaur S, Ovrehus KA, Dey D, et al. Coronary plaque quantification and fractional flow reserve by coronary computed tomography angiography identify ischaemia-causing lesions. *Eur Heart J* 2016; 37: 1220–1227.
  23. Dey D, Achenbach S, Schuhbaeck A, et al. Comparison of quantitative atherosclerotic plaque burden from coronary CT angiography in patients with first acute coronary syndrome and stable coronary artery disease. *J Cardiovasc Comput Tomogr* 2014; 8: 368–374.
  24. Polonsky TS, McClelland RL, Jorgensen NW, et al. Coronary artery calcium score and risk classification for coronary heart disease prediction. *JAMA* 2010; 303: 1610–1616.
  25. Erbel R, Mohlenkamp S, Moebus S, et al. Coronary risk stratification, discrimination, and reclassification improvement based on quantification of subclinical coronary atherosclerosis: the Heinz Nixdorf Recall study. *J Am Coll Cardiol* 2010; 56: 1397–1406.
  26. de Araujo Goncalves P, Garcia-Garcia HM, Carvalho MS, et al. Diabetes as an independent predictor of high atherosclerotic burden assessed by coronary computed tomography angiography: the coronary artery disease equivalent revisited. *Int J Cardiovasc Imaging* 2013; 29: 1105–1114.
  27. Krul MM, Bogaard K, Knol RJ, et al. Coronary artery disease in patients with atypical chest pain with and without diabetes mellitus assessed with coronary CT angiography. *BMJ Open Diabetes Res Care* 2014; 2: e000004.
  28. Tofterup ML, Beck-Nielsen H, Gerke O, et al. One-third of patients with type 2 diabetes mellitus do not have coronary artery calcification. *J Prev Med Care* 2015; 1: 1–8.
  29. Leber AW, Knez A, Becker A, et al. Accuracy of multi-detector spiral computed tomography in identifying and differentiating the composition of coronary atherosclerotic plaques: a comparative study with intracoronary ultrasound. *J Am Coll Cardiol* 2004; 43: 1241–1247.
  30. Amano T, Matsubara T, Uetani T, et al. Abnormal glucose regulation is associated with lipid-rich coronary plaque: relationship to insulin resistance. *JACC Cardiovasc Imaging* 2008; 1: 39–45.
  31. Burke AP, Kolodgie FD, Zieske A, et al. Morphologic findings of coronary atherosclerotic plaques in diabetics: a postmortem study. *Arterioscler Thromb Vasc Biol* 2004; 24: 1266–1271.
  32. Virmani R, Burke AP and Kolodgie F. Morphological characteristics of coronary atherosclerosis in diabetes mellitus. *Can J Cardiol* 2006; 22: 81B–84B.
  33. Motoyama S, Sarai M, Harigaya H, et al. Computed tomographic angiography characteristics of atherosclerotic plaques subsequently resulting in acute coronary syndrome. *J Am Coll Cardiol* 2009; 54: 49–57.
  34. Motoyama S, Ito H, Sarai M, et al. Plaque characterization by coronary computed tomography angiography and the likelihood of acute coronary events in mid-term follow-up. *J Am Coll Cardiol* 2015; 66: 337–346.
  35. Nakanishi R, Ceponiene I, Osawa K, et al. Plaque progression assessed by a novel semi-automated quantitative plaque software on coronary computed tomography angiography between diabetes and non-diabetes patients: a propensity-score matching study. *Atherosclerosis* 2016; 255: 73–79.
  36. Fok PW. Growth of necrotic cores in atherosclerotic plaque. *Math Med Biol* 2012; 29: 301–327.
  37. Edsfeldt A, Goncalves I, Grufman H, et al. Impaired fibrous repair: a possible contributor to atherosclerotic plaque vulnerability in patients with type II diabetes. *Arterioscler Thromb Vasc Biol* 2014; 34: 2143–2150.
  38. Lehman SJ, Schlett CL, Bamberg F, et al. Assessment of coronary plaque progression in coronary computed tomography angiography using a semiquantitative score. *JACC Cardiovasc Imaging* 2009; 2: 1262–1270.
  39. Machida H, Tanaka I, Fukui R, et al. Current and novel imaging techniques in coronary CT. *Radiographics* 2015; 35: 991–1010.
  40. He T, Qian X, Zhai R, et al. Computed tomography number measurement consistency under different beam hardening conditions: comparison between dual-energy spectral computed tomography and conventional computed tomography imaging in phantom experiment. *J Comput Assist Tomogr* 2015; 39: 981–985.