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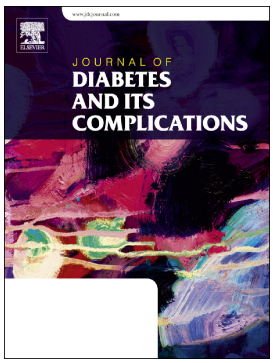

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Coronary artery calcifications and diastolic dysfunction versus visceral fat area in type 1 diabetes: VISCERA study.

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ABSTRACT:

Aims: Type 1 diabetic patients (T1DM) experience a higher cardiovascular disease and mortality risk than controls. We investigated whether visceral adipose tissue (VAT) contributes to coronary artery calcifications (CAC) and cardiac dysfunction in T1DM.

Methods: A cross-sectional study of 118 T1DM patients without a history of cardiovascular disease (men/women: 68/50, age 46±12 y, HbA1c 7.6±0.9%, BMI 25.8±4.1 kg/m²) was conducted. CAC and VAT were measured using a CT scan. CAC was scored using the Agatston method. Cardiac functional abnormalities were assessed by echocardiography.

Results: CAC scored ≥10 in 42% of patients. Systolic function was normal in all, but diastolic dysfunction was present in 75%. Forty-six percent had VAT≥100 cm^2. CAC score≥10 occurred more often in subjects with VAT≥100 cm^2 (54% vs 31%,p=0.01). Age (OR=1.10;p<0.0001), diabetes duration (OR=1.10;p=0.008), gender (OR=4.28;p=0.016), LDL-cholesterol (OR=1.03;p=0.009) and metabolic syndrome (OR=5.79;p=0.005) were independently associated with a CACS≥10. Subjects with CACS≥10 were more prone to have diastolic dysfunction (84 vs 54%; p=0.03). Factors independently associated with diastolic dysfunction were age (OR=1.11;p=0.002), waist circumference (OR=1.10;p=0.016) and VAT (OR=0.99;p=0.035).

Conclusions: Excess VAT in T1DM, present in 46%, is associated with diastolic dysfunction and CAC, present in respectively 75% and 42% of patients. Timely detection might improve future cardiovascular risk.

Keywords: type 1 diabetes; coronary artery calcifications; diastolic dysfunction; visceral fat; adipocytokines

ClinicalTrials.gov Identifier: NCT02689570

1. INTRODUCTION:

Patients with type 1 diabetes (T1DM) experience a higher incidence of cardiovascular disease (CVD) and a 3 times -times higher mortality risk for men and 7.5x increased risk for women compared to age-matched controls (de Ferranti et al., 2014; Libby et al., 2005; Livingstone et al., 2012; Cleary et al., 2006). Coronary artery disease (CAD) and heart failure caused by cardiomyopathy are the leading causes of death. Among young asymptomatic T1DM patients, the incidence of CAD is 1-2% (Orchard et al., 2003; Soedamah-Muhtu et al. 2008). Early signs of coronary atherosclerosis can be quantitated by measuring coronary artery calcifications (CAC) (Olson et al. 2000). In T1DM patients, the presence and extent of CAC may even predict CAD events (Olson et al., 2000; Dabelea et al., 2003). However, diabetes not only affects the vasculature but also the myocardial tissue. Diastolic dysfunction is an early sign of diabetic cardiomyopathy, appearing as early as after 6-8 years of diabetes (Shivalkar et al., 2006; Kuznetsova et al., 2009). It preceeds the onset of systolic dysfunction by up to 10 years (Kuznetsova et al., 2009; Suys et al., 2004) and is associated with poor prognosis (Halley et al., 2011).

The excess risk of CVD in T1DM can not only be explained by classic risk factors such as HbA1c, dyslipidemia, hypertension, and smoking (Orchard et al., 2003; Olson et al. 2000; Nathan et al., 2005), but increased visceral adipose tissue (VAT) may also contribute (Olson et al. 2000; Van Gaal et al., 2006; Conway et al., 2007). Low plasma concentrations of adiponectin have been linked to progression of CAC in T1DM, independently of other CVD risk factors (Maahs et al., 2005), but the association with leptin, tumour necrosis factor alpha (TNF-α) or interleukin-6 (IL-6) has not been studied before.

Since more T1DM subjects are overweight today (De Block et al. 2005), we ascertained the prevalence of subclinical cardiac abnormalities by scoring CAC and by performing

echocardiography, and investigated a possible link with VAT and adipocytokines. The high prevalence and impact of CAD and heart failure in T1DM underscore the need to identify CVD risk factors at an early, subclinical stage in these patients. This might allow timely prevention of future CVD.

2. METHODS:

In this cross-sectional study, 118 adult T1DM patients, aged 18-75 years, regularly attending the out-patient diabetes clinic of the Antwerp University Hospital, were recruited between June 2011 and February 2013. All patients fulfilled the criteria for the diagnosis of T1DM established by the American Diabetes Association. Patients had to have a diabetes duration of ≥5 years and be in good general health. Exclusion criteria were a history of myocardial infarction, angina pectoris, stroke, amputation, heart failure NYHA class III-IV, pregnancy or a glomerular filtration rate ≤30 ml/min/1.73 m^2. All participants signed an informed consent form. This study was conducted in accordance with the amended Declaration of Helsinki. The research protocol was approved by the Antwerp University Hospital Ethics committee (UZA 11/31/224, Belgian registration number: B30020111874).

2.1. Anthropometric measurements:

All measurements were performed in the morning after an overnight fast. Height was measured using a wall-mounted stadiometer, and weight was measured using a digital scale with subjects in their underwear. Waist circumference was measured at the mid-level between the lower rib margin and the iliac crest. VAT and subcutaneous abdominal adipose tissue (SAT) were determined by a 64-slice CT scan at the L4–L5 level (slice thickness 0.6mm) (Van der Kooy et al. 1993).

Blood pressure was taken with a standardized method (Dynamap) after a 10-min rest, and a mean of five measurements was used. Hypertension was defined as blood pressure ≥130/85 mmHg or antihypertensive medication intake, using the same criteria as for the metabolic syndrome (Alberti et al., 2009). Presence of the metabolic syndrome was defined if ≥3 of the following criteria were met: increased waist circumference (men ≥94 cm, women ≥80 cm), hypertriglyceridemia (≥150 mg/dl=1.7 mmol/l), low HDL-cholesterol (men <40 mg/dl=1.03 mmol/l, women <50 mg/dl=1.29 mmol/l), blood pressure ≥130/85 mmHg, fasting glucose ≥100 mg/dl.

Insulin resistance was determined using the inverse of the estimated glucose disposal rate (eGDR), calculated using the formula: eGDR = 21.158 + (- 0.09 * waist circumference) + (- 3.407 * hypertension) + (- 0.551 * HbA1c) (Epstein et al., 2013). We also used a newer equation, eliminating HbA1c from the formula, in order to better distinguish between estimated insulin sensitivity and glycemic control; eIS = exp (4.06154 − 0.01317 * waist [cm] − 1.09615 * insulin dose [daily units per kg] + 0.02027 * adiponectin [μg/mL] − 0.00307 * triglycerides [mg/dL] − 0.00733 * DBP [mmHg]) (Duca et al., 2016).

2.2. Laboratory measurements:

HbA1c was determined by high performance liquid chromatography (Adams™ A1c HA- 8180, Arkray–Menarini instrument, Zaventem, Belgium; reference range: 4.8-6.0%). A mean of four annual determinations of HbA1c was used to assess overall metabolic control over the past two years. Serum levels of creatinin, total cholesterol, HDL cholesterol and triglycerides were measured on a Dimension Vista 1500 System (Siemens Healthcare Diagnostics, Huizingen, Belgium) with reagents from the same manufacturer (respectively REF K1270A, K1027, K3048A, K2069).

The glomerular filtration rate (eGFR) was estimated using the abbreviated MDRD formula. Plasma HMW adiponectin concentrations were measured by ELISA (EZHMWA-64K, Millipore, analytical sensitivity 0.5 ng/ml, intra-assay coefficient of variation [CV] 7.47%). Leptin was measured using an ELISA kit (HU Leptin kit, KAC2281, Life Technologies, sensitivity 3.5 pg/ml, intra-assay CV 5.75%). TNF-α was assayed using ELISA (HU TNF alpha kit, KHC3011, Life Technologies, sensitivity 1.7 pg/ml, intra-assay CV 6.38%) and IL-6 concentrations were measured using ELISA (HU IL-6 Chemiluminescence Elisa kit, KHC0069, Life Technologies, sensitivity 0.25 pg/ml, intra-assay CV 5.37%).

2.3. Assessment of complications:

Each subject was assessed for presence and severity of complications (De Block et al., 2005). 24h-Urinary albumin was measured by nephelometry (Dimension Vista 1500 System, Siemens Healthcare Diagnostics, Huizingen, Belgium; reagent REF K7062). Microalbuminuria was defined as a urinary albumin excretion >20 µg/min. Both carotid arteries were examined using a carotid duplex scanner (General Electrics, Vivid 7 Pro), equipped with an 8-Mhz high-resolution probe. A 64-slice non-contrast multidetector CT scan of the coronary arteries was performed to measure CAC (Lightspeed, VCT; General Electric Medical Systems, Waukesha, Wis, Milwaukee, USA). Scoring was done by one skilled radiologist (R.S.), who was blinded to the subjects case files. Typical imaging parameters were: tube voltage 100 kv; current intensity 310 mA; rotation time 500 ms; and detector collimation 64 x 0.625 mm. Scan data were reconstructed at 75% of the cardiac cycle after the QRS complex. The radiation dose for calcium scoring ranged at 1.3–1.7 mSv. CAC was quantified (Agatston score) by means of a dedicated software application (SmartScore, AW). The Agatston score is calculated by multiplying the area of each calcified lesion with a weighted CT attenuation score dependent on the maximal CT-attenuation

(Hounsfied Units) within the lesion. A score <10 was considered as normal (Wexler et al., 1996). A score ≥200 is a strong predictor of CVD risk (Cleary et al., 2006); Lachin et al., 2014).

Standard 2-dimensional and Doppler Echocardiography (iE-33 Philips, The Netherlands) was performed by a single cardiologist blinded to the clinical status of the study participants. Left ventricular systolic function was assessed by modified biplane Simpson method. Diastolic function was determined and classified as grade 1 to 4 taking into account all the following parameters: mitral inflow (early rapid filling wave, late filling wave, deceleration time, isovolumetric relaxation time), pulmonary vein inflow signal and mitral annular tissue Doppler (tD) velocities from end expiratory cycles. Pulse-wave tD systolic (Sm) and diastolic (early, Em; late, Am) velocities were obtained from the apical 4-, 3-, and 2-chamber views from end expiratory cycles. Signals were obtained from three end expiratory cycles, and averages were made for the systolic and diastolic velocities. An Em/Am ratio of <1 was considered to represent abnormal segmental diastolic function for patients aged <60 years (Wilkenshoff et al., 2001).

2.4. Statistical analysis:

Data are expressed as mean ± standard deviation for normally distributed continuous variables and median (interquartile range: 25^{th}-75^{th} percentile) for non-normally distributed continuous variables. The normality of distribution of continuous variables was assessed with the Kolmogorov-Smirnov method. Variables that were not normally distributed were log transformed or square rooted when appropriate. Comparison between two groups were performed using the unpaired t-test or the Mann-Whitney U test when appropriate, with Bonferroni adjustments for multiple comparisons. Differences in distributions of categorical data were evaluated by Chi square or Fisher Exact test. Correlations between variables were performed using the Pearson or Spearman's rank test when appropriate. Linear regression analysis was used to examine

parameters associated with logVAT as dependent variable. Multiple logistic regression analysis was applied to examine parameters independently associated with a CAC score≥10, and with diastolic dysfunction.

LASSO (least absolute shrinkage and selection operator logistic regression) was applied because it performs both variable selection and regularization in order to enhance the prediction accuracy and interpretability of the statistical model it produces (Tibshirani, 1996). In case of small sample sizes, LASSO is beneficial, because it puts restrictions on the absolute size of the regression coefficients. It reports best subset selection that best predicts the outcome and the connections between LASSO coefficient estimates and so-called soft thresholding. It also reveals that (like standard logistic regression) the coefficient (the regression coefficients represent the change in the logit for each unit change in the predictor) estimates need not be unique if covariates are collinear. The amount of restriction is tuned by a leave-one-out cross validation mean squared error prediction (Meier et al., 2008)

A two-tailed P-value <0.05 was considered significant. Statistical analyses were performed with SPSS Statistics software, version 22.0 (Armonk, NY, USA).

3. RESULTS:

The study population comprised 118 T1DM patients (68 men) with a mean age of 46±12 years and diabetes duration of 25±10 years. HbA1c averaged 7.7±0.8% (61±9 mmol/mol) over the last two years. BMI averaged 25.8±4.1 kg/m² and mean waist circumference was 94±12 cm for men and 85±13 cm for women. The mean VAT measurements were 130±81 cm^2 and 82±54 cm^2 respectively. Only 11% of patients achieved an LDL-cholesterol <70 mg/dl. Fifty patients were smokers of whom 10 had stopped smoking. Hypertension was present in 47 patients (40%). Forty-nine patients (42%) had the metabolic syndrome. Median coronary artery calcifications

score (CACS) was 196 (range 0-3405). CACS was ≥10 in 42% of patients, while 17% had a CACS ≥200. Systolic function was normal in all (LVEF >55%), but diastolic dysfunction was present in 88 patients (75%).

3.1. Characteristics comparing subjects with a VAT ≥100 cm^2 versus VAT <100 cm^2:

Fifty-four (46%) T1DM patients had a VAT ≥100 cm^2. They were older than subjects with VAT<100 cm^2, but diabetes duration and HbA1c levels were similar (see Table 1). Men had more VAT than women (121 (interquartile range: 61-181) vs 67 (46-100) cm^2, p<0.001). Mean insulin requirements were higher in subjects with VAT≥100 cm^2 (p=0.008), and estimated glucose disposal rate (eGDR) and estimated insulin sensitivity (eIS), both estimates of insulin sensitivity, were lower (both p<0.001). The metabolic syndrome was also more prevalent (61 vs 25%; OR=4.71; p<0.001), and lipid lowering and antihypertensive drugs were more often used in patients with VAT≥100 cm^2 than in those with VAT <100 cm^2. Adiponectin levels were lower (p=0.002) and leptin (p=0.012) and TNF-α levels higher (p=0.022) in the group with VAT ≥100 cm^2.

Univariate analysis showed correlations between VAT and age (r=0.41,p<0.0001), BMI (r=0.58,p<0.0001), waist circumference for men (r=0.55,p<0.0001) and women (r=0.67,p<0.0001), adiponectin (r=-0.20,p=0.044), leptin (r=0.23,p=0.003), LDL-cholesterol (r=0.19,p=0.041), triglycerides (r=0.27,p=0.004), systolic blood pressure (r=0.25,p=0.002), diastolic blood pressure (r=0.19,p<0.043), eGDR (r=-0.56,p<0.0001), insulin sensitivity (eIS) (r=-0.39,p<0.0001) and CACS (r=0.27,p=0.003).

Microalbuminuria was equally present in both groups, but glomerular filtration rate was lower in patients with VAT ≥100 cm^2 (p=0.04). Moreover, CAC score was higher in subjects with VAT ≥100 cm^2 (p=0.024), being ≥10 in 54% of these subjects versus 31% in the group with a VAT

<100 cm^2 (OR=2.66,p=0.01). The number of subjects with diastolic dysfunction was similar between groups. Linear regression analysis showed that eIS (estimated insulin sensitivity) was the only factor independently associated with logVAT (R^2=0.36, β= -0.068 ±0.023, p=0.011).

3.2. Characteristics comparing subjects with a CACS ≥10 versus <10:

CAC score was ≥10 in 49 subjects (M/F=34/15,OR=2.27,p=0.039). These patients were older (p<0.001), had a longer diabetes duration (p<0.001), higher BMI (p=0.001), larger waist circumference and higher VAT (p<0.0001) than those with a CACS<10 (Table 2). The metabolic syndrome was also more prevalent (65 vs 24%, OR=6.12, p<0.001) and lipid lowering and antihypertensive drugs were more frequently used in patients with CACS≥10. HbA1c levels did not differ. Estimated glucose disposal rate (eGDR) was lower in subjects with a CACS≥10 (p<0.0001). No differences in adiponectin, IL-6, leptin or TNF-α concentrations were observed.

Glomerular filtration rate was modestly lower (p=0.04), but microalbuminuria did not differ between groups. Macrovascular abnormalities were also more prevalent in this group as evidenced by more frequent carotid plaques (37% vs 13%, OR:6.67, p<0.001), wall motion abnormalities (16 vs 4%; OR=4.07; p=0.03), and diastolic dysfunction (84 vs 54%, OR=4.07; p=0.03).

Univariate analysis showed correlations between CACS and age (r=0.41,p<0.0001), waist circumference for men (r=0.42,p<0.0001) and women (r=0.29,p=0.02), VAT (r=0.27,p=0.003) and eGDR (r=-0.33,p<0.0001). Figure 1 shows the percentage of T1DM patients per age-group and gender with a CACS≥10 and per VAT quartile. Logistic regression analysis identified age (OR=1.10; 95% CI=1.04-1.17; p<0.0001), diabetes duration (OR=1.10; 95% CI= 1.03-1.17; p=0.008), gender (OR=4.28; 95% CI=1.38-13.24; p=0.016), LDL-cholesterol (OR=1.03; 95%

CI=1.01-1.05; p=0.009), presence of the metabolic syndrome (OR=5.79; 95% CI=1.84-23.31; p=0.005), and use of lipid-lowering drugs (OR=3.59; 95% CI 1.12-10.74; p=0.039), but not HbA1c, VAT, systolic blood pressure as parameters independently associated with a CACS≥10. In a model where metabolic syndrome was replaced by eGDR, factors independently associated with a CACS≥10 were age (OR=1.12; 95% CI=1.06-1.21; p=0.001), diabetes duration (OR=1.09; 95% CI=1.03-1.20; p=0.009), gender (OR=3.72; 95% CI=1.02-11.46; p=0.023), and eGDR (OR=0.77; 95% CI=0.61-0.96; p=0.025). Further analyses showed that the factors independently associated with a CACS≥10 were not significantly different between men and women. In women, these independent factors were age (OR=1.16;p=0.013) and metabolic syndrome (OR=2.56; p=0.022), whereas in men, these were age (OR=1.14;p=0.009), diabetes duration (OR=1.16;p=0.012) and metabolic syndrome (OR=2.03;p=0.07).

LASSO identified age, diabetes duration, waist circumference, systolic blood pressure, LDL-cholesterol, eGDR, and visceral fat area as independent predictors in the optimal model for CACS (penalty=0.300). From these factors, age (β=0.254,p<0.001), diabetes duration (β=0.019,p=0.978) and waist circumference (β=0.062,p=0.466) were included in the selected model of LASSO for CACS (R^2=0.338,p<0.001,penalty=0.580).

3.3. **Characteristics comparing subjects with versus without diastolic dysfunction:**

Patients with diastolic dysfunction were older (p<0.001), had a longer diabetes duration (p<0.001), a lower eGDR (p=0.016), and men had a larger waist circumference (p=0.021) as compared to those with a normal diastolic function at rest and at peak stress (Table 3). There were no differences in gender, HbA1c, blood pressure, lipids, smoking status, presence of metabolic syndrome, and adipocytokines between those with and without diastolic dysfunction. No association with diastolic dysfunction was observed when VAT was classified in a

dichotomous way (using 100 cm^2 as cutoff), in contrast to when VAT was evaluated as a continuous parameter. Indeed, logistic regression analysis identified age (OR=1.11, 95% CI=1.04-1.18; p=0.002), waist circumference (OR=1.10; 95% CI=1.02-1.19; p=0.016) and VAT (OR=0.99; 95% CI=0.97-0.99; p=0.035), but not diabetes duration, gender, systolic blood pressure, LDL cholesterol, HbA1c, metabolic syndrome, eGDR, eIS, smoking, or CAC score, as factors independently associated with diastolic dysfunction.

LASSO was performed to select the best model of factors independently associated with diastolic dysfunction and identified age and diabetes duration to be included. From these factors, diabetes duration (β=0.021,p=0.978) was the only factor included in the selected model of LASSO for diastolic dysfunction (R^2=0.130,p<0.001,penalty=0.740).

4. **DISCUSSION**:

Despite the fact that the risk of cardiovascular mortality is 3 times higher for men and 7.5x higher for women compared to age-matched controls (de Ferranti et al., 2014; Libby et al., 2005; Livingstone et al., 2012; Cleary et al., 2006), identification of subclinical cardiac abnormalities such as CAC and diastolic dysfunction is presently not included in diabetes complications surveillance programs. Visceral fat accumulation and insulin resistance are likely to contribute to this increased risk (Conway et al., 2007). Moreover, T1DM patients are not spared from the epidemic of overweight (De Block et al., 2005).

Among our apparently healthy T1DM patients, 46% had a VAT≥100 cm^2, diastolic dysfunction was present in 75%, and 42% had a CACS ≥10, while 17% had a CACS ≥200. In the intensively treated group of the DCCT/EDIC cohort, with similar age, diabetes duration and HbA1c, but higher BMI, 8.5% had a CACS ≥200 (Lachin et al., 2014). Age, diabetes duration, gender, and presence of metabolic syndrome or eGDR, but not HbA1c or smoking status were independently

associated with a CACS ≥10 in our study. LASSO identified age, diabetes duration and LDL-cholesterol as being the best predictors. The factors independently associated with a CACS≥10 were not significantly different between men and women.

Others have also found age and diabetes duration to be the strongest correlates of CAC (Cleary et al., 2006; Dabelea et al., 2003; Maahs et al., 2005). A positive association between waist circumference and presence of CAC has been reported before (Cleary et al., 2006; Dabelea et al., 2003). Conway et al. observed a link between VAT and presence but not extent of CAC in T1DM (Conway et al., 2007). Adiposity may contribute to CAD via increased lipolysis of VAT with release of adipocytokines into the systemic circulation, and excess free fatty acids into the portal vein. An increased flux of free fatty acids to the liver will increase triglyceride and small-dense LDL-cholesterol synthesis, and may induce insulin resistance, while IL-6 has been linked to CAC (Alman et al., 2013). Low adiponectin levels are associated with VAT accumulation and cardiovascular mortality in T1DM (Maahs et al., 2005; Forsblom et al., 2011). However, we could not observe a statistical difference in adiponectin levels between those with a CACS≥10 versus those with a lower CACS. A link between leptin and CAC has been observed in non-diabetic and in T2DM subjects (Qasim et al., 2008), but no data exist in T1DM, except for this study. Despite a negative correlation between eGDR, a marker of insulin sensitivity, and leptin, we did not observe significant differences in leptin levels between patients with or without CACS≥10.

Features of the metabolic syndrome may help explain the increased prevalence of CAC in T1DM. In the Pittsburgh EDC cohort, 8% of subjects (Pambianco et al., 2007), in the FinnDiane Study 36% (Thorn et al., 2009), and in our cohort 42% met the diagnostic criteria. We observed the metabolic syndrome to be an independent predictor of a CACS≥10. In the DCCT/EDIC study, however, where 22% of patients had the metabolic syndrome, this syndrome did not predict

micro- or macrovascular events over 17 years of follow-up (Kilpatrick et al., 2007). Dichotomous classification of T1DM subjects according to the metabolic syndrome appears to add no value for CV risk prediction over and above its individual components. A role for insulin resistance contributing to CAC (Dabelea et al., 2003, Bjornstad et al., 2016) and a higher CAD risk in T1DM patients has been proposed (Olson et al., 2002). Our results show an inverse relationship between eGDR and CAC severity, and confirm these observations (Orchard et al., 2003; Olson et al., 2002).

HbA1c, an important predictor of microvascular complications, does not seem to play a major role in macrovascular complications. Some studies showed a link between glycemic control and CAC (Cleary et al., 2006; Lachin et al., 2014), whereas others did not (Orchard et al., 2003; Olson et al., 2000; Nathan et al., 2005). In the DCCT study, intensive diabetes therapy and the attendant 6.5 years of lower HbA1c were associated with thinner carotid intima media thickness, less CAC and a 57% lower incidence of major cardiovascular events, providing a strong argument pro early good glycemic control (Lachin et al., 2014). However, the DCCT cohort was selected to exclude patients with dyslipidemia and hypertension on study entry, in contrast to our T1DM population. Without the influence of these conventional risk factors, the impact of HbA1c might have become more apparent. Also, most of our patients showed an acceptable to good glucose control, with a too small range between those with lowest to highest HbA1c to detect significant differences. In our study, the use of antihypertensive or lipid-lowering drugs was not independently associated with presence of CAC.

Diabetic nephropathy may amplify the risk of CVD in T1DM patients (Maahs et al., 2013).In contrast, T1DM adults free of nephropathy even present no excess mortality compared to the general population as noted in the FinnDiane (Groop et al., 2009) and the EDC study (Orchard et al., 2010). Conway et al. observed that CAC severity was linked to GFR (Conway et al., 2007).

We and others (Thilo et al., 2004) could not find an association between microalbuminuria and CAC, but we did not include patients with end-stage renal disease. On the other hand, GFR was lower in subjects with VAT≥100 cm^2 and they were also more likely to have a CACS≥10, despite the more frequent use of lipid-lowering and antihypertensive drugs. Scoring of CAC seems to be the most powerful cardiac risk predictor in asymptomatic subjects, with consistent superiority to traditional risk factors (Cleary et al., 2006; Lachin et al., 2014). Our results show that subjects with CACS≥10 were more prone to have plaques at the carotid arteries. In the DCCT/EDIC trial, CAC correlated with carotid IMT that was measured 1-3 years earlier (Cleary et al., 2006). A meta-analysis including 8 studies with 6521 T2DM individuals showed a 5.47x increased risk for all cause mortality or cardiovascular events for patients with CACS≥10 (Kramer et al., 2013). However, data in T1DM are missing. In T2DM patients with minimal (CACS<10) or no CAC, excellent 5-year survival (99%), not being different from the non-diabetic population, was demonstrated. Based on these observations, a high-risk group could be identified for whom intensive therapy, including statin therapy, would be indicated. However, except for the St Francis Heart Study in asymptomatic individuals with a positive family history for premature CAD and elevated CACS, no data exist. In that randomized trial, comprising >90% non-diabetic subjects, atorvastatin lowered CAD events in subjects with CACS≥400, with non-significant reduction of events in those with lower CACS (Arad et al., 2005).

Diabetes not only affects the vasculature, but also the myocardial tissue. Diastolic dysfunction in the absence of hypertension or CAD is the earliest manifestation of diabetic cardiomyopathy (Kuznetsova et al., 2009; Wai et al., 2014) and precedes the onset of systolic dysfunction (Kuznetsova et al., 2009; Suys et al., 2004). Data from the FLEMENGHO study in Belgium show that myocardial function is affected much earlier in T1DM as compared to the control

population (Kuznetsova et al., 2009). According to a register-based survey in Sweden, the incidence of heart failure in patients with T1DM aged 41-45 years is equal to that in individuals from the general population aged 55-64 years (Lind et al., 2011). In our study, diastolic dysfunction was present in 75% of T1DM subjects, which is comparable with the recently reported 69% (Wai et al., 2014). Lind et al. observed that each unit increase in BMI represented a 5% increased risk of hospitalization for heart failure in patients with T1DM (Lind et al., 2011), whereas the risk rose by 30% with each 1% increase in HbA1c. Data from the DCCT/EDIC study show that HbA1c was positively correlated with LV mass and negatively correlated with stroke volume and ejection fraction (Genuth et al., 2013). Age, waist circumference and VAT were identified as independent factors for diastolic dysfunction using logistic regression, whereas diabetes duration was identified as being the best predictor using LASSO. The association with VAT was marginal with an OR of 0.99, probably because the group of patients with a normal diastolic function was very small, the range of VAT was large (with overlap between groups) and more than half of the patients with diastolic dysfunction had a VAT < 100 cm^2. The association with waist circumference was more solid and in the expected direction. Diastolic dysfunction is associated with increased mortality (Halley et al., 2011). Whether avoiding overweight or accumulation of VAT will translate into an improved future cardiovascular outcome is unknown. However, since both Lind and we observed an association between diastolic dysfunction and VAT, weight control might be important.

4.1. Study limitations and strengths:

Our study is subject to selection bias, as only ambulatory patients in good general health without a history of overt CVD of chronic kidney disease were included. Therefore, the true prevalence of subclinical CVD is probably underestimated. Furthermore, this is a cross-sectional analysis

precluding any causality. Nevertheless, the association between VAT, eGDR and early cardiovascular abnormalities we observed, lends support to the idea of VAT being an important modulator of cardiovascular risk in T1DM patients.

Strengths of this study is its the meticulous methodology used. We are the first to investigate the links between VAT and its adipocytokines and insulin resistance on the one hand, and the combination of CAD and diastolic dysfunction on the other hand in T1DM.

4.2. Conclusion and proposal:

Considering that patients with T1DM are younger, they are at risk of losing many more life-years from CVD than T2DM patients. We have clearly shown that excess VAT in T1DM, present in nearly half of the patients, is linked to early signs of macrovascular disease. Diastolic dysfunction was present in 75% and CAC in >40% of our patients. Therefore, we suggest to incorporate screening for subclinical CVD using CACS and echocardiography into the diabetes surveillance programs of chronic complications. This approach may provide incremental prognostic information over traditional risk stratification to guide the intensity of primary prevention treatments. Strategies including early identification of risk factors including VAT, and aggressive treatment of subclinical disease for patients at high risk for future overt CAD or heart failure, may favourably affect outcome. Whether prevention of weight gain in the context of diet and lifestyle, or the use of weight-lowering or insulin-sensitizing drugs could improve prognosis in type 1 diabetes, should be further investigated.

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Authors' Contributions:

All authors made important intellectual contributions to the conception and design of the study. Every author reviewed and provided comments on manuscript drafts and gave final approval of this version to be published. L.V.G. was responsible for the final design of the protocol. C.D.B. and L.V.G. recruited patients. All authors implemented the study protocol and acquired data. C.D.B., W.G., T.B., K.C. and A.V. performed the statistical analysis. C.D.B., W.G., T.B., K.C. and L.V.G. drafted the manuscript.

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Figure Legends:

Figure 1A: Percentage of T1DM patients with a CACS≥10, per age and gender.

Figure 1B: Percentage of T1DM patients with a CACS≥10, per quartile of VAT and per gender.

Table 1: Characteristics comparing subjects with a VAT <100 cm^2 versus VAT ≥100 cm^2

	VAT < 100 cm^2	VAT ≥ 100 cm^2	p-value	univariate correlation with VAT	Unadjusted Odds ratio (95% CI)	Adj rat
n (M/F)	64 (26/38)	54 (42/12)	<0.001		5.11 (2.27 - 11.53)	7.31
cardiovascular risk factors						
age (y)	45 (31-54)	52 (42-59)	0.001	r:0.41/p<0.001		
duration of T1DM (y)	23 (18-30)	25 (21-35)	NS	r:0.20/p=0.029		
HbA1c (%)	7.7 ± 0.9	7.7 ± 0.8	NS			
HbA1c (mmol/mol)	60 ± 9	60 ± 8	NS			
insulin (U/kg BW)	0.66 ± 0.22	0.78 ± 0.23	0.008	r:0.39/p<0.001		
BMI (kg/m^2)	23.8 ± 3.5	28.1 ± 3.7	0.001	r:0.58/p<0.001		
waist circumference men (cm)	85 (77-90)	98 (93-106)	<0.001	r:0.55/p<0.001		
waist circumference women (cm)	79 (74-86)	103 (90-110)	<0.001	r:0.67/p<0.001		
metabolic syndrome (n)	16 (25%)	33 (61%)	<0.001	r:0.42/p<0.001	4.71 (2.15 – 10.36)	3.92
blood pressure systolic (mmHg)	124 (118-130)	130 (124-136)	0.004	r:0.25/p=0.002		
blood pressure diastolic (mmHg)	75 (69-80)	80 (73-80)	NS	r:0.19/p=0.043		
total cholesterol (mg/dl)	190 ± 33	189 ± 34	NS			
HDL cholesterol (mg/dl)	71 ± 23	58 ± 15	<0.001	r:-0.27/p=0.004		
LDL cholesterol (mg/dl)	100 ± 24	109 ± 32	NS	r:0.19/p=0.041		
triglycerides (mg/dl)	87 ± 39	103 ± 73	NS	r:0.27/p=0.004		
smoking (n)	27 (42%)	23 (43%)	NS			
lipid-lowering drugs (n)	11 (18%)	24 (45%)	0.001		3.91 (1.68 – 9.12)	4.80
antihypertensive drugs (n)	14 (23%)	25 (51%)	0.002		3.50 (1.54 – 7.93)	3.17
eGDR (mg/kg per min)	9.1 (6.8-10.1)	5.4 (4.3-7.9)	<0.001			
insulin sensitivity (eIS)	5.4 (4.3-7.2)	3.4 (2.6-4.4)	<0.001			
macrovascular complications						

CACS (Agatston score)	0 (0-40)	19 (0-99)	0.024	r:0.27/p=0.003		
CACS ≥ 10 (n)	20 (31%)	29 (54%)	0.01		2.66 (1.25 – 5.66)	2.55
carotid artery plaques (n)	12 (19%)	15 (28%)	NS			
wall motion abnormalities (n)	4 (6%)	7 (13%)	NS			
diastolic dysfunction (n)	47 (73%)	41 (76%)	NS			
microvascular complications						
microalbuminuria (µg/min)	11 (7-14)	9 (6-15)	NS			
retinopathy (n)	23 (36%)	27 (50%)	NS			
polyneuropathy (n)	20 (31%)	17 (31%)	NS			
glomerular filtration rate (ml/min/1,73 m^2)	90 (89-90)	90 (80-90)	0.045			
adipocytokines						
IL-6 (pg/ml)	0.13 (0.13-3.22)	1.32 (0.13-4.24)	NS			
TNF-α (pg/ml)	16.3 (13.8-19.6)	18.0 (15.4-22.9)	0.022			
adiponectin (ng/ml)	12222 (6961-21463)	6089 (4217-14265)	0.002	r:-0.20/p=0.044		
leptin (pg/ml)	8698 (3002-18073)	11837 (7055-27211)	0.012	r:0.23/p=0.003		

Legend: Data are presented as numbers, as mean ± standard deviation, or as median (interquartile range: 25^{th}-75^{th} percentiles). CACS: coronary artery calcification score; eGDR: estimated glucose disposal rate, eIS: estimated insulin sensitivity; VAT: visceral adipose tissue.

Table 2: Characteristics comparing subjects with a CACS<10 versus CACS≥10

	CACS < 10	CACS ≥ 10	p-value	univariate correlation with CACS	Unadjusted Odds ratio (95% CI)	Adju rati
n (M/F)	69 (34/35)	49 (34/15)	0.039		2.27 (1.05-4.90)	4.
cardiovascular risk factors						
age (y)	40 (31-50)	55 (51-60)	< 0.001	r:0.41/p<0.001	1.15 (1.09-1.21)	1.10
duration of T1DM (y)	22 (14-27)	31 (22-37)	< 0.001	r:0.37/p<0.001	1.12 (1.07-1.18)	1.10
HbA1c (%)	7.6 ± 0.9	7.7 ± 0.9	NS			
HbA1c (mmol/mol)	59 ± 12	63 ± 12	NS			
insulin (U/kg BW)	0.70 ± 0.17	0.73 ± 0.29	NS	r:0.29/p<0.003		
BMI (kg/m^2)	24.7 ± 4.1	27.3 ± 3.8	0.001	r:0.17/p=0.069		
waist circumference men (cm)	90 (77-98)	96 (92-106)	< 0.001	r:0.42/p<0.001	1.08 (1.04-1.12)	
waist circumference women (cm)	79 (74-88)	89 (78-108)	0.033	r:0.29/p=0.044		
visceral adipose tissue (VAT) (cm^2)	66 (41-131)	117 (77-185)	< 0.001	r:0.27/p=0.003		
VAT ≥ 100 cm^2	25 (36%)	29 (59%)	0.01		2.66 (1.25-5.66)	
metabolic syndrome (n)	16 (24%)	33 (67%)	< 0.001	r:0.23/p=0.015	3.40 (1.54-7.49)	5.
blood pressure systolic (mmHg)	124 (118-130)	130 (125-137)	< 0.001			
blood pressure diastolic (mmHg)	77 (70-80)	78 (71-80)	NS			
total cholesterol (mg/dl)	185 ± 33	195 ± 33	NS			
HDL cholesterol (mg/dl)	66 ± 23	63 ± 19	NS			
LDL cholesterol (mg/dl)	100 ± 26	111 ± 31	0.03		1.01 (1.00-1.03)	1.03
triglycerides (mg/dl)	90 ± 39	99 ± 77	NS			
smoking (n)	26 (38%)	24 (49%)	NS			
lipid-lowering drugs (n)	10 (14%)	25 (51%)	< 0.001		6.60 (2.68 – 16.21)	3.
antihypertensive drugs (n)	17 (25%)	22 (45%)	0.009		2.94 (1.30 – 6.66)	
eGDR (mg/kg per min)	8.8 (6.6-10.1)	5.4 (4.4-8.2)	< 0.001	r:-0.33/p<0.001		
insulin sensitivity (eIS)	4.8 (3.6-4.8)	3.7 (2.6-6.0)	NS			
macrovascular complications						
CACS (Agatston score)	0 (0-0)	103 (42-565)	< 0.001			
carotid artery plaques (n)	9 (13%)	18 (37%)	< 0.001		6.67 (2.38 – 18.63)	

wall motion abnormalities (n)	3 (4%)	8 (16%)	0.03		4.07 (1.01 – 16.3)
diastolic dysfunction (n)	47 (54%)	41 (84%)	0.03		4.07 (1.09 – 15.17)
microvascular complications					
microalbuminuria (µg/min)	10 (7-15)	10 (6-14)	NS		
retinopathy (n)	24 (35%)	26 (53%)	0.03		2.34 (1.07 – 5.08)
polyneuropathy (n)	20 (29%)	17 (35%)	NS		
glomerular filtration rate (ml/min/1,73 m^2)	90 (88-90)	90 (81-90)	0.04	r:-0.23/p=0.014	
adipocytokines					
IL-6 (pg/ml)	1.77 (0.13-5.15)	0.13 (0.13-2.27)	NS		
TNF-α (pg/ml)	17.1 (14.4-21.4)	17.9 (14.7-20.8)	NS		
adiponectin (ng/ml)	10962 (4967-17729)	9443 (5124-24018)	NS		
leptin (pg/ml)	8460 (5277-17762)	11934 (5756-27211)	NS		

Legend: Data are presented as numbers, as mean ± standard deviation, or as median (interquartile range: 25^{th}-75^{th} percentiles). CACS: coronary artery calcification score; eGDR: estimated glucose disposal rate, eIS: estimated insulin sensitivity; VAT: visceral adipose tissue.

Table 3: Characteristics comparing subjects without versus with diastolic dysfunction:

	nl diastolic function	diastolic dysfunction	p-value	Unadjusted odds ratio (95% CI)	Adjusted O ratio (95%
n (M/F)	17 (18/12)	89 (50/38)	NS		
cardiovascular risk factors					
age (y)	32 (29-45)	50 (40-56)	<0.001	1.10 (1.04-1.16)	1.11 (1.04-1
duration of T1DM (y)	16 (7-22)	25 (20-35)	<0.001		
HbA1c (%)	7.7 ± 0.6	7.7 ± 0.7	NS		
HbA1c (mmol/mol)	59 ± 8	60 ± 8	NS		
insulin (U/kg BW)	0.70 ± 0.22	0.74 ± 0.29			
BMI (kg/m^2)	23.9 ± 3.5	26.0 ± 4.1	0.053 (NS)		
waist circumference men (cm)	83 (74-92)	94 (88-99)	0.021		
waist circumference women (cm)	79 (73-89)	86 (75-93)	NS	1.07 (1.01-1.12)	1.10 (1.02-1
visceral adipose tissue (VAT) (cm^2)	69 (43-131)	94 (50-158)	NS	1.00 (0.996-1.01)	0.99 (0.9 0.999)
VAT ≥ 100 cm^2	13 (43%)	41 (47%)	NS		
metabolic syndrome (n)	7 (23%)	42 (48%)	0.053 (NS)		
blood pressure systolic (mmHg)	125 (116-132)	128 (120-133)	NS		
blood pressure diastolic (mmHg)	77 (69-80)	76 (70-80)	NS		
total cholesterol (mg/dl)	184 ± 33	190 ± 35	NS		
HDL cholesterol (mg/dl)	66 ± 22	64 ± 21	NS		
LDL cholesterol (mg/dl)	100 ± 33	106 ± 29	NS		
triglycerides (mg/dl)	87 ± 40	97 ± 64	NS		
smoking (n)	12 (40%)	38 (43%)	NS		
lipid-lowering drugs (n)	5 (17%)	30 (34%)	NS		
antihypertensive drugs (n)	8 (27%)	31 (35%)	NS		
eGDR (mg/kg per min)	9.1 (6.3-10.6)	7.3 (5.1-9.2)	0.016		
insulin sensitivity (eIS)	5.2 (2.6-6.5)	4.6 (3.4-6.0)	NS		
macrovascular complications					
CACS (Agatston score)	0 (0-0)	4 (0-103)	0.01		
CACS ≥ 10 (n)	8 (27%)	41 (47%)	<0.001		
carotid artery plaques (n)	4 (13%)	23 (26%)	NS		
wall motion abnormalities (n)	1 (3%)	10 (11%)	NS		

microvascular complications			
microalbuminuria (μg/min)	8 (6-11)	10 (6-15)	NS
retinopathy (n)	9 (30%)	41 (47%)	NS
polyneuropathy (n)	6 (20%)	31 (35%)	NS
glomerular filtration rate (ml/min/1,73 m^2)	90 (90-90)	90 (83-90)	NS
adipocytokines			
IL-6 (pg/ml)	0.75 (0.13-6.04)	0.18 (0.13-3.70)	NS
TNF-α (pg/ml)	15.5 (13.3-21.6)	17.4 (14.6-21.4)	NS
adiponectin (ng/ml)	7500 (3581-17446)	11028 (5098-20412)	NS
leptin (pg/ml)	8974 (2667-20920)	10779 (5648-19549)	NS

Legend: Data are presented as numbers, as mean ± standard deviation, or as median (interquartile range: 25^{th}-75^{th} percentiles). CACS: coronary artery calcification score; eGDR: estimated glucose disposal rate, eIS: estimated insulin sensitivity; VAT: visceral adipose tissue.

Figure 1A: Percentage of T1DM patients with a CACS≥10, per age and gender.

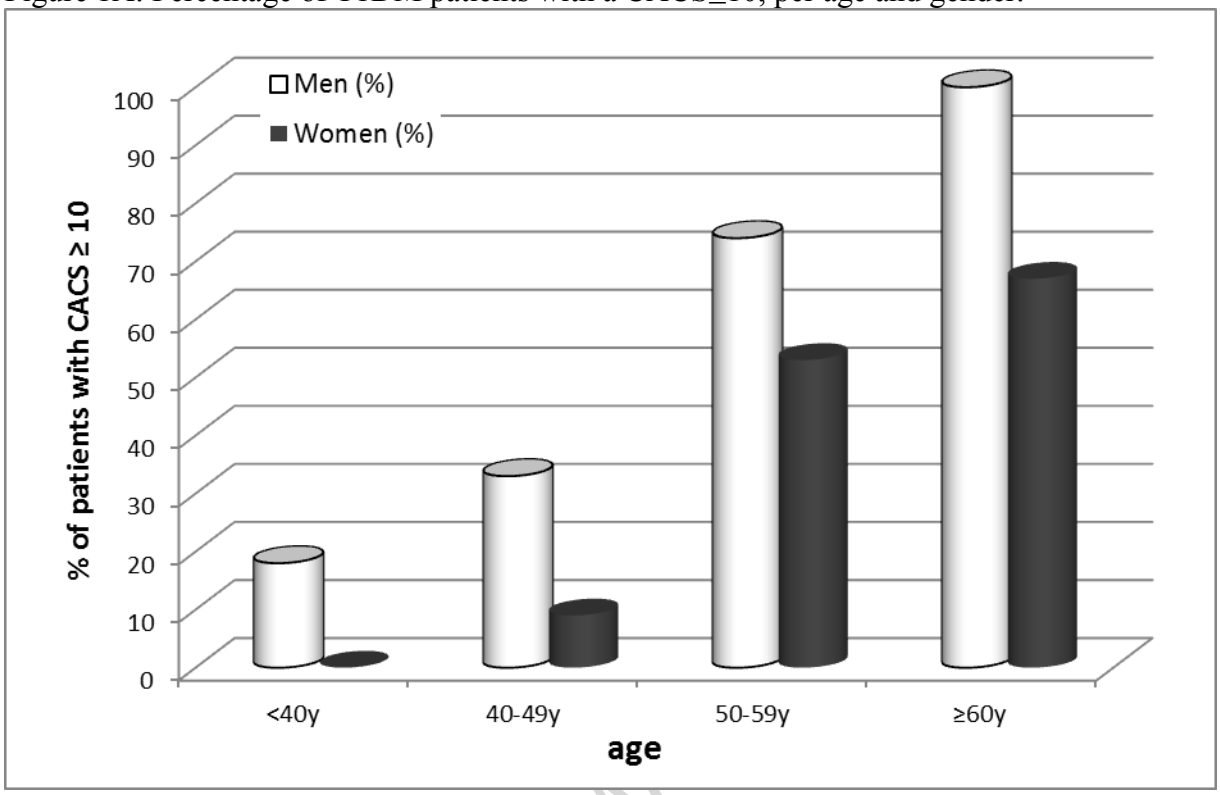

Fig 1B: Percentage of T1DM patients with a CACS≥10, per quartile of VAT and per gender.