Title:

Ectopic and visceral fat deposition in lean and obese type 2 diabetes patients

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Abstract

BACKGROUND- Type 2 diabetes (T2D) and obesity are associated with non-alcoholic fatty liver disease, cardiomyopathy and cardiovascular mortality. Both conditions showed stronger links between ectopic and visceral fat deposition and increased cardio-metabolic risk than subcutaneous fat deposition.

OBJECTIVES- We tested the hypothesis that even lean patients with T2D (Ln-T2D) exhibit increased ectopic and visceral fat deposition, and that these are linked to cardiac and hepatic changes.

METHODS- Twenty-seven obese (Ob-T2D), fifteen Ln-T2D with no other comorbidities, and twelve age matched controls were studied. Patients underwent cardiac CT (epicardial fat volume quantification), cardiac MRI (volumes and function), ^{31}P-MRS (myocardial PCr/ATP), ^{1}H-MRS (myocardial triglyceride) and multi-parametric liver MRI (^{1}H-MRS hepatic triglyceride; T1- and T2*-mapping yielding 'iron-corrected T1' [cT1], a measure of hepatic fibrosis and inflammation). Healthy, lean subjects underwent identical MRI protocols.

RESULTS- Diabetes, even in the absence of obesity, was associated with increased myocardial triglyceride content (p=0.01), hepatic triglyceride content (p=0.04) and impaired myocardial energetics (p=0.04). While cardiac structural changes, steatosis and energetics were similar between the T2D groups, epicardial fat (p=0.04), hepatic triglyceride (p=0.01) and insulin resistance (p=0.03) were higher in Ob-T2D. Epicardial fat, hepatic triglyceride and insulin resistance correlated negatively with systolic strain and diastolic strain rates which were only significantly impaired in Ob-T2D (p<0.001 and p=0.006, respectively). Fibroinflammatory liver disease (elevated cT1) was only evident in Ob-T2D (p=0.004 and

p<0.001 vs Ln-T2D and controls, respectively). cT1 correlated with hepatic and epicardial fat (p<0.001 and p=0.01 respectively).

CONCLUSIONS- Irrespective of BMI, diabetes is related to significant abnormalities in cardiac structure, energetics, cardiac and hepatic steatosis. Obese patients with T2D show a greater propensity for ectopic and visceral fat deposition that is associated with cardiac dysfunction, fibroinflammatory liver disease and insulin resistance.

Key Words:

- Diabetic cardiomyopathy
- Epicardial fat deposition
- Fatty liver disease
- Magnetic resonance imaging
- Magnetic resonance spectroscopy

Introduction

Type 2 diabetes (T2D) and obesity are both associated with non-alcoholic fatty liver disease (NAFLD), cardiomyopathy(1,2), and increased cardiovascular mortality(3,4). The incidence of T2D continues to increase and is driven predominantly by the obesity epidemic. Although obesity is likely to be a strong contributor to diabetic cardiomyopathy(5), many patients with diabetic cardiomyopathy have normal body mass index (BMI), suggesting that diabetes and obesity may have different mechanisms by which they mediate cardiovascular change, and that diabetic cardiomyopathy may occur in patients with T2D without obesity. Furthermore, accumulating evidence suggests that the distribution of excess fat is an important determinant of cardiovascular risk, and ectopic and visceral adiposity confer a much higher risk than subcutaneous adiposity(6,7).

Ectopic and visceral fat storage may be linked to insulin resistance, and it is widely known that insulin resistance is the strongest predictor of development of diabetes(8). Importantly, increasing evidence points to a strong association between insulin resistance and nonischemic heart failure(9), albeit with differing opinions held whether this relationship is of protective or pathological nature(10-12). Thus, the presence of ectopic and visceral fat deposition in patients with T2D even in the absence of a global increase in total body fat may potentially play a significant role in this association. Assessing body composition is therefore likely to be more important in patients with T2D than simple metrics of obesity. Accordingly, there is growing interest in the imaging of epicardial adipose tissue as a proxy measure of visceral fat and liver fat, which is considered to be a key feature of ectopic fat associated with dysfunctional adipose tissue and visceral fat deposition(13).

Epicardial adipose tissue, which is a form of visceral fat, may affect the biology of the underlying myocardium, by secreting a wide range of adipokines(14). Furthermore, excess

liver fat, which is a form of ectopic fat, has been shown to be accompanied by cardiac structural and functional changes(15). Computed tomography (CT) allows assessment of epicardial fat volume quantification, and ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) allows quantification of lipid content in the liver and the heart. Multi-parametric magnetic resonance of the liver, including ^{1}H-MRS for assessment of steatosis and T1 and T2* mapping (yielding iron corrected T1-cT1)(16), allows not only non-invasive quantification of liver fat, but also identification of the presence of hepatic fibroinflammatory disease with a high diagnostic accuracy(16).

Myocardial energetic compromise is an important feature of both the diabetic (17) and the non-diabetic obese heart(5). However, changes in cardiac energy metabolism in lean diabetic patients have not been studied. Myocardial phosphocreatine to ATP concentration ratio (PCr/ATP) is a sensitive indicator of the myocardial energy status and phosphorus magnetic resonance spectroscopy (^{31}P-MRS) allows non-invasive assessment of the PCr/ATP. The primary aim of this study was to test the hypothesis that even lean patients with T2D (Ln-T2D) exhibit increased ectopic and visceral fat deposition. Our secondary aim was to test whether or not the ectopic and visceral adiposity in diabetes are associated with insulin resistance, cardiac and hepatic changes. With these aims we used cardiac CT, multi-parametric liver MRI, and cardiac MRI, ^{1}H-MRS , Phosphorus-MRS (^{31}P-MRS) to assess and compare epicardial, hepatic and myocardial fat deposition, hepatic fibroinflammatory changes, and cardiac structure, function, and energetics in lean and obese patients with T2D (Ob-T2D), and non-diabetic controls.

Methods

The study was approved by the National Research Ethics Committee (REC Ref 13/SW/0257) and informed written consent was obtained from each participant. Patients were recruited from general practice surgeries in Oxfordshire, United Kingdom. Twenty-seven Ob-T2D, fifteen Ln-T2D and twelve healthy normal weight controls were recruited to the study. We have previously reported changes in myocardial energetics and triglyceride content, and left ventricular structure and function in diabetic patients compared to healthy volunteers (18,19). Taking advantage of this database, and expansion of the data with novel recruitment of 12 healthy volunteers to the study, we now report a comparison of the changes in these cardiac features in two subgroups of diabetes patients (obese and lean diabetics) compared to healthy volunteers. Additionally, we now report an analysis of epicardial fat volumes, liver triglyceride content and liver fibroinflammatory changes.

Exclusion Criteria

Subjects were excluded if they had a previous diagnosis of cardiovascular or liver disease, tobacco smoking, hypertension (resting systolic blood pressure (BP) >140 mmHg and diastolic BP >90 mmHg), contraindications to MR imaging, ischemic changes on 12-lead ECG, renal impairment (estimated glomerular filtration rate below 30 mL/min), alcohol intake above 21 units in a week for men or 14 units for women, or if they were insulin dependent.

Controls had no history of heart disease, diabetes mellitus (fasting glucose level ≥6.7 mmol), hypertension, and were not taking any medications.

Study assessments were carried out on a single visit for the healthy controls, and over two or three visits for T2D-patients, depending on individuals' consent for attending cardiac CT assessments (Figure 1).

Anthropometric Measurements

Height and weight were recorded and BMI calculated. Brachial blood pressure was recorded as an average of 3 supine measures taken over 10 minutes (DINAMAP-1846-SX, Critikon Corp). Fasting venous blood was drawn for glucose, insulin, HbA1c, triglyceride, renal function, liver function, free fatty acids (FFA). Insulin levels and HbA1c were checked in the diabetes patients, not in controls. Homeostasis model assessment of insulin resistance (HOMA-IR) was used to evaluate insulin resistance (fasting serum insulin (μU/L) × fasting plasma glucose (mmol l^{-1})/22.5)(20).

Cardiac Computed Tomography

Coronary Computed Tomographic Angiography

An optional scan of coronary computed tomographic angiography (CCTA) was offered to diabetic patients to exclude obstructive coronary artery disease (>50% of luminal stenosis) and for assessment of epicardial fat volumes. CCTA was performed with a GE VCT 64 slice scanner (GE Healthcare) and a Snapshot Pulse protocol with prospective ECG triggering. Participants received beta-blockade (intravenous Metoprolol) and sublingual GTN prior to the scan to achieve a heart rate of <65 beats per minute. During the CCTA acquisition, 70ml of iodinated contrast (Niopam 370, Bracco, UK) was injected at a rate of 6ml/sec followed by a 50ml saline flush. The scan covered a region from 1-2cm above the left main coronary artery to 1-2cm below the myocardial apex in a single breath hold.

Epicardial Fat Volume Quantification

CT images were reconstructed using medium-soft kernel (standard) with slice thickness of 0.625mm and then transferred to a dedicated workstation for image processing, TeraRecon

Aquarius iNtuition (version 4.4.11 TeraRecon Inc. San Mateo, CA). The adipose tissue volume was quantified using contrast enhanced CT images. The layer of the epicardium was manually traced and using semi-automated method a 3D image constructed. The volume was then calculated by a blinded operator (ST) and defined as the tissue with attenuation of -190 to -30 Hounsfield Units.

Cardiac Magnetic Resonance

All LV imaging was performed on a 3.0 Tesla MR system (Siemens, Germany). Images for LV volumes and diastolic function were acquired using a steady state free precession (SSFP) sequence and analysed using cmr42© (Circle Cardiovascular Imaging Inc, Canada).

To determine mid-ventricular systolic circumferential strain and diastolic strain rate, myocardial tagging was performed(21,22). Tagged images were analysed using Cardiac Image Modeller software (CimTag2D v7 Auckland Medical Research, Auckland, New Zealand). Semi-automated analysis was performed by aligning a grid to the myocardial tagging planes at end-diastole. A more detailed description of the MRI methods and MR acquisition parameters is included in the supplementary methods.

^{31}P-MR Spectroscopy

^{31}P MR spectroscopy was performed to obtain the rest PCr/ATP from a voxel placed in the mid-ventricular septum, with the subjects lying prone with their heart over the centre of the ^{31}P heart/liver coil in the isocentre of the magnet. ^{31}P-MRS post processing analysis was performed using in house software within Matlab version R2012a (Mathworks, Natick, Massachusetts). A more detailed description of the cardiac ^{31}P-MR Spectroscopy acquisition parameters is included in the supplementary methods.

Cardiac and Liver ^{1}H-MRS

Myocardial ^{1}H-MR spectra were obtained from the mid-interventricular septum. Liver triglyceride content was measured using ^{1}H MRS, avoiding vascular and biliary structures. Spectroscopic acquisitions were performed using ECG trigger. Water-suppressed spectra were acquired to measure myocardial and liver triglyceride content, and spectra without water suppression were acquired and used as an internal standard. Spectra were analyzed using Matlab and the AMARES algorithm in Java-based Magnetic Resonance User Interface. Myocardial and liver triglyceride contents were calculated as a percentage relative to water: (signal amplitude of lipid/signal amplitude of water)×100. A more detailed description of the cardiac ^{1}H-MR Spectroscopy acquisition parameters is included in the supplementary methods.

Liver Magnetic Resonance Imaging

The liver multi-parametric MR protocol has been previously described(16). MR scans were performed using a 3-Tesla scanner (Tim Trio, Siemens Healthcare, Germany). Transverse abdominal T1 and T2* MR maps were acquired for the estimation of extracellular fluid and liver iron respectively. Patients attended for their MRI scans after fasting overnight.

Iron corrected T1 and Fibroinflammatory Liver Disease

T1 relaxation time increases with increases in extracellular fluid such as in fibrosis and inflammation. However, the presence of iron, which can be accurately measured from T2* maps, has an opposing effect on the T1. An algorithm has been created that allows for the

bias introduced by elevated iron to be removed from the T1 measurements, yielding the iron corrected T1 (cT1).

LiverMultiScan^{TM} (LMS, Perspectum Diagnostics, Oxford, UK), is a software product, developed specifically to measure cT1 from T1 and T2* maps. For this study, LMS was used to analyse anonymised images, by investigators blinded to the clinical data. cT1 was measured in a single, operator-defined, region of interest away from vascular and biliary structures.

Statistical Analysis

All statistical analysis was performed with commercially available software packages (IBM SPSS Statistics, version 20). All data were checked for normality using Kolmogorov-Smirnov test and presented as mean ± standard deviations. Comparisons between the 3 groups were performed by 1-way ANOVA with post hoc Bonferroni corrections. Bivariable correlations were performed using the Pearson or Spearman method as appropriate. Student *t* test was used for comparison of normally distributed data sets where data were obtained for only two T2D groups. Significance was defined as $P<0.05$.

Results

Participant Characteristics

Demographic, clinical, and biochemical data are shown in Table 1.

Twenty-seven Ob-T2D patients (14 male, mean age 56 ± 8years, BMI 33 ± 3kg/m^2 , mean diabetes duration 6.1 ± 4.7years, mean HbA1c of 7.7 ± 1.4%), fifteen Ln-T2D–patients (9

male, mean age 56 ± 9years, BMI 23 ± 2kg/m^2, mean diabetes duration of 6.6 ± 6.5years, mean HbA1c of 7.4 ± 0.9%) and twelve healthy volunteers (8 male, mean age 50 ± 10years, BMI 23 ± 2kg/m^2) were recruited. Participants in all groups were of similar age, gender, and there were no significant differences in blood pressure, diabetes duration, diabetes treatment or metabolic profile between the two diabetes groups (Table 1). Systolic blood pressure was statistically higher in participants with T2D compared to controls, although remained within normal limits. 77% of the diabetics were on statin therapy, and patients with diabetes therefore had lower low-density lipoprotein cholesterol levels compared to controls.

Cardiac Geometry and Function

CMR results for LV volumes and function are summarized in Table 2. LV volumes and ejection fraction were similar between lean and Ob-T2D, and controls. However, while LV ejection fractions were not significantly different across the groups, more subtle functional changes with impairment in peak circumferential systolic strain and diastolic strain rates were evident in Ob-T2D compared to controls (p=0.001 and p=0.006, respectively) and also compared to Ln-T2D (p=0.015, p=0.026, respectively). As we have previously shown, diabetes was associated with LV concentric hypertrophy(19), as defined by increased LV mass to volume ratio and increased LV mass, in both diabetes groups compared to controls.

Cardiac Metabolic Phenotype

Cardiac ^{1}H- and ^{31}P-MRS results for myocardial triglyceride and energetics are summarized in Table 2.

Diabetes was associated with cardiac steatosis even in the absence of obesity (Ln-T2D vs controls, p=0.01) and strikingly there was no significant difference in myocardial triglyceride levels between the obese and Ln-T2D. PCr/ATP was significantly reduced in both T2D groups compared to controls (Ob-T2D vs controls, p=0.002; Ln-T2D vs controls, p=0.043). There was no significant difference in myocardial PCr/ATP ratio between the Ob-T2D and the Ln-T2D (p=0.92). There were no significant correlations between the myocardial PCr/ATP ratio and the markers of ectopic and visceral adiposity, such as hepatic triglyceride content (r=-0.17, p=0.36), or with epicardial fat volume (r=-0.23, p=0.27).

Epicardial Fat

Epicardial fat volume assessment was carried out in 33 patients (79% of the study patients) who have opted for CCTA. Ob-T2D had higher epicardial fat volumes compared to Ln-T2D (96 ± 40cm^3 vs 71 ± 21cm^3 , p=0.04). Figure 3 shows representative images of epicardial fat volume in a lean and an Ob-T2D patient.

Hepatic Steatosis, Iron Content, Fibrosis and Inflammation

Liver enzymes and multiparametric liver MRI results for hepatic steatosis, fibrosis and haemosiderosis are summarized in Table 3. Similar to cardiac steatosis, diabetes, even in the absence of obesity, was associated with hepatic steatosis (hepatic triglyceride content in Ln-T2D vs controls, p=0.044); however, hepatic steatosis was most marked in Ob-T2D, approximately two-fold higher compared to Ln-T2D (Ob-T2D vs Ln-T2D, p=0.012), and approximately four-fold higher compared to controls (Ob-T2D vs controls, p=0.005). Iron levels were normal across the groups.

Mean cT1 was highest in the Ob-T2D, where the highest levels of hepatic triglyceride content were detected. The numeric differences in mean cT1 between Ln-T2D and controls did not reach statistical significance (p=0.245), while cT1 in the Ob-T2D was significantly increased compared to Ln-T2D (p=0.004) and controls (p<0.001), indicating significant fibroinflammatory liver disease in this group. There was a positive correlation between the hepatic cT1 and hepatic triglyceride content (r=0.71, p<0.001). Importantly, despite the presence of hepatic steatosis and fibroinflammatory changes in the Ob-T2D group, there was no significant difference in liver enzymes, compared to controls, and there was no association between liver cT1 and liver enzymes. Alanine aminotransferase (ALT) levels were only minimally elevated (>45IU/L, <80IU/L) in 5 Ob-T2D and were normal in all other patients.

Figure 4 shows representative liver ^{1}H-MR spectra, a liver T1 map and cardiac tagging in a volunteer, a lean and an obese T2D patient.

Relationship between Insulin Resistance, Ectopic Fat Accumulation and Cardiac Function

Insulin resistance, measured by HOMA-IR, was significantly higher in Ob-T2D compared to Ln-T2D (p=0.03). When investigating all T2D subjects, there was a positive correlation between the HOMA-IR and epicardial fat volumes (r=0.47, p=0.029), hepatic triglyceride (r=0.39, p=0.046) and with hepatic cT1 (r=0.58, p=0.001); and a negative correlation between HOMA-IR and peak circumferential systolic strain (r=-0.52, p=0.003). Furthermore, peak circumferential systolic strain also correlated negatively with the hepatic triglyceride (r=-0.49, p=0.001) and epicardial fat volumes (r=-0.53, p=0.004). Similarly, diastolic strain rate correlated negatively with hepatic triglyceride (r=-0.54, p<0.001) and epicardial fat

volumes (r=-0.59, p=0.001). Myocardial triglyceride did not correlate with epicardial fat volumes (r=0.36, p=0.103), or with hepatic triglyceride (r=0.23, p=0.168).

Discussion

This study demonstrates for the first time that diabetes, even in the absence of obesity, is associated with significant cardiac structural and metabolic abnormalities, while significant functional changes such as reductions in peak systolic strain and diastolic strain rates are only evident in obese diabetics. Furthermore we show that those patients with diabetes who are also obese have higher epicardial fat volumes, significant NAFLD, and higher insulin resistance. Importantly, we demonstrate here that the degree of hepatic and epicardial fat accumulation is associated with cardiac contractile dysfunction in diabetes. We confirm the findings of previous studies showing the association between epicardial fat deposition and insulin resistance(23,24); moreover, we also demonstrate that there is an association between fibroinflammatory liver disease and insulin resistance in patients with diabetes. The correlation of systolic strain and diastolic strain rates with hepatic and epicardial fat and insulin resistance in diabetic patients suggest a link between these; however, the causality of these relationships will need to be investigated in future studies.

Finally, as widely known, the spectrum of NAFLD ranges from fatty liver alone to nonalcoholic steatohepatitis (NASH) (25). We show here that diabetes, even in the absence of obesity, is associated with hepatic steatosis, at the mild end of the liver disease spectrum, but not with significant fibroinflammatory liver disease. Importantly, using multiparametric liver imaging, we show that significant NAFLD and NASH are present in asymptomatic T2D patients with minor or no ALT elevation in diabetics. This technique promises to answer a

pressing need for a reliable, quick, non-invasive screening, staging and monitoring tool for diabetic liver disease.

Ectopic and visceral fat, insulin resistance and the heart

Our results suggest that Ln-T2D patients are likely to have less pronounced insulin resistance, lower levels of epicardial and hepatic fat accumulation and better cardiac function than obese diabetic patients. It is now widely accepted that adipose tissue is a dominant regulator of lipid and glucose metabolism(26). Multiple studies support the concept that insulin resistance is prompted, and sustained by, dysregulated fat tissue(27-29). In addition, insulin resistance and ectopic adiposity are associated with an even greater cardiovascular risk(30,31) and obese subjects with T2D are at high risk of developing ectopic adiposity(32). There are many molecular mechanisms that may contribute to the association between insulin resistance and non-ischemic cardiomyopathy(9). These include metabolic inefficiency(33), impaired vascular function(34), inflammation, mitogenic actions of insulin on myocardium leading to changes of left ventricular geometry(35).

Epicardial adipose tissue has dichotomous functional characteristics, both adverse and protective, interacting locally with the coronary arteries and the myocardium through paracrine and vasocrine pathways. Under physiological conditions, epicardial fat supplies heat to the myocardium and exerts a protective effect on the coronary arteries(23,36). Its pathological increase, and the co-existence of other metabolic and hemodynamic abnormalities, turn it into an adverse lipotoxic, prothrombotic and proinflammatory organ(31).

In our study, the dissociation of myocardial steatosis from epicardial and liver fat is in keeping with a previous study in non-diabetics, and supporting the hypothesis that myocardial lipid accumulation may represent a separate entity that is influenced by factors beyond visceral adiposity(37). Rijzewijk et al and McGavock et al previously demonstrated myocardial steatosis in patients with T2D(38,39). Furthermore, McGavock et al performed ^{1}H-MRS in a large cohort of T2D patients and were first to show that hepatic triglyceride was not predictive of myocardial triglyceride(38), thus showing elevated levels of intracellular triglyceride in hepatocytes do not necessarily reflect elevated triglyceride levels in cardiac myocytes in T2D, which we confirm here.

Ectopic fat and the liver

Our results suggest that Ln-T2D patients are more likely to have simple steatosis and Ob-T2D patients are more likely to have steatohepatitis. Our study is the first to date to non-invasively assess the severity of liver damage using a multi-parametric MRI protocol in lean and Ob-T2D patients and the impact of fibroinflammatory liver disease on the cardiac phenotype. We have demonstrated that asymptomatic Ob-T2D patients have significantly higher liver cT1 compared to Ln-T2D patients and healthy volunteers. This would indicate a greater burden of fibro-inflammatory liver disease in this group of patients who should be prioritised for NAFLD screening in clinical practice. Importantly, these differences were present on imaging but not on ALT levels, suggesting that ALT alone is not a sensitive screening test for the presence of NAFLD in these patients. It has previously been shown that liver cT1 is associated with fibrosis(16) and also that it can differentiate simple steatosis from steatohepatitis(40-42).

NAFLD is defined as the excessive fat accumulation in the liver (>5.6%) (43) and it is among the leading causes of death in T2D(44) and linked to hepatic insulin resistance(45). Despite this strong association and the emergence of NAFLD as a novel cardiovascular risk factor, only a few studies have addressed the presence of myocardial structural and functional changes in patients with NAFLD. Specifically, NAFLD, diagnosed either by ultrasonography or by liver biopsy, was shown to be associated with a higher prevalence of reduced coronary flow reserve(46), coronary calcification(47), impairment in diastolic function(48), concentric LV remodelling, and reduced longitudinal shortening(49).

Limitations

This study is limited by a relatively small sample size, in line with its proof-of-principle nature.

Of the 42 patients with T2D, 9 patients (21%) did not consent to have CCTA performed and as such it is possible that occult coronary artery disease could be present in this minority of patients. CCTA was not performed in the normal volunteers to avoid unnecessary radiation exposure. Significant coronary artery disease was deemed to be unlikely in this normal cohort and epicardial fat volumes were therefore only assessed and compared in obese and lean T2D patients.

For the assessment of the liver disease we have not used liver biopsy, the current gold standard, because it is invasive and limited by sampling and observer dependent variability(50), instead we have used a recently established, non-invasive multi-parametric scanning method, which has been demonstrated to have a high diagnostic accuracy for the assessment of liver fibrosis, steatosis and haemosiderosis(16).

Although the numeric differences in mean peak systolic strain and diastolic strain rates in Ln-T2D compared to controls did not reach statistical significance this may be due to relatively small sample size. Larger studies of patients with lean T2D needed to confirm this.

Whilst the release of various adipokines including adiponectin and leptin has been considered among the important actions of adipocytes, in our study we have not assessed the circulating levels of adiponectin or leptin.

There is evidence suggestive of a role for the sympathetic nervous system in the relation between insulin and hypertension in hypertensive obese patients(51). Although we have not demonstrated any significant difference in resting heart rates or the systolic blood pressure between the diabetic patients to suggest enhanced adrenergic drive in the obese group in this study, we have not assessed circulating cathecholamine levels.

Finally, although our findings of the significant correlations between insulin resistance, cardiac function, epicardial and hepatic fat, and hepatic fibroinflammation are striking, their observational nature precludes inferences of causality. Additional research is necessary to further delineate the relationship between ectopic and visceral adiposity with potential systemic effects such as insulin resistance and their role in the development of cardiac dysfunction in patients with T2D.

Conclusions

Irrespective of BMI, diabetes is related to significant abnormalities in cardiac function, energetics, and cardiac and hepatic steatosis. However, obese patients with T2D show a

greater propensity for ectopic fat deposition that is associated to cardiac contractile dysfunction and fibroinflammatory liver disease.

CLINICAL PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Irrespective of BMI, diabetes is related to significant abnormalities in cardiac structure, energetics, cardiac and hepatic steatosis. Obese patients with diabetes show a greater propensity for ectopic fat deposition that is associated with cardiac dysfunction, fibroinflammatory liver disease and insulin resistance. Significant non-alcoholic fatty liver disease and non-alcoholic steatohepatitis are present in asymptomatic type 2 diabetes patients with minor or no ALT elevation, highlighting a pressing need for a reliable, quick, non-invasive screening, staging and monitoring tool for diabetic liver disease.

TRANSLATIONAL OUTLOOK 1: Larger studies are needed to further delineate the relationship between ectopic and visceral adiposity with potential systemic effects such as insulin resistance and their role in the development of cardiac dysfunction in patients with type 2 diabetes.

TRANSLATIONAL OUTLOOK 2: As a truly quantitative non-invasive technique for the characterisation of liver tissue, the multiparametric MRI liver protocol has the potential for the safe longitudinal assessment and prediction of diabetic liver disease progression, regression, and/or response to therapy, without the need for repeat liver biopsy.

Disclosures

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M.P., S.N., M.R. and C.K. are shareholders in Perspectum Diagnostics and have patents in the field of MR for the assessment of liver disease. S.N. is also a non-executive director of Perspectum Diagnostics.

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Table 1: Clinical and Biochemical Characteristics

Variable	Normal Controls N=12	Lean T2D patients N=15	Obese T2D patients N=27	P value
Age, y	50 ± 10	56 ± 9	56 ± 8	0.163
BMI, kg/m^2	23 ± 3	23 ± 2	33 ± 3†	<0.001
Male,%	58	60	40	0.35
Diabetes duration, y	…	6.1 ± 4.7	6.6 ± 6.5	0.78
Heart rate, bpm	66 ± 10	65 ± 7	69 ± 7	0.34
Systolic blood pressure, mmHg	118 ± 14	131 ± 7*	130 ± 9*	0.002
Diastolic blood pressure, mmHg	70 ± 8	76 ± 7	76 ±7	0.05
Plasma fasting glucose, mmol/L	5.0 ± 0.5	8.1 ± 3.0*	9.5 ± 3.3*	0.001
Glycated hemoglobin, %	…	7.4 ± 0.9	7.7 ± 1.4	0.22
Hematocrit, %	43 ± 3	42 ± 3	43 ± 3	0.81
Insulin, pmol/L	…	107 ± 142	218 ± 255	0.03
HOMA-IR, %	…	1.26 ± 0.70	5.45 ± 5.6	0.03
Plasma triglycerides, mmol/L	0.92 ± 0.38	1.87 ± 1.81	1.75 ± 0.81	0.15
Plasma free fatty acids, mmol/L	0.59 ± 0.42	0.61 ± 0.20	0.67 ± 0.43	0.82
Total cholesterol, mmol/L	4.7 ± 1.0	3.8 ± 0.8	4.1 ± 1.0	0.10
HDL, mmol/L	1.55 ± 0.56	1.24 ± 0.29	1.20 ± 0.31*	0.03
LDL, mmol/L	2.93 ± 0.46	1.85 ± 0.59*	2.12 ± 0.82*	0.002
Medications, n (%)				
Metformin, n (%)	…	14 (93)	23 (85)	0.45
Sulphonylurea	…	4 (27)	12 (44)	0.27
Aspirin	…	2 (13)	7 (26)	0.35
Statin	…	8 (60)	19 (70)	0.51
ACE-I	…	7 (47)	10 (37)	0.56

Values are mean ± standard deviations or percentages. T2D indicates type 2 diabetes; BMI, body mass index; n, number; y, years; bpm, beats per minute; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; ACE-I angiotensin-converting enzyme inhibitors.

*P<0.05 vs controls, with Bonferroni Correction

†P<0.05 vs lean T2D and controls with Bonferroni Correction

Table 2: CMR and Cardiac MRS Findings

	Controls N=12	Lean T2D patients N=15	Obese T2D patients N=27	P value
LV end-diastolic volume, ml	145 ± 40	124 ± 33	126 ± 25	0.15
LV ejection fraction, %	68 ± 5	73 ± 7	68 ± 8	0.11
LV mass, g	91 ± 30	123 ± 33*	119 ± 28*	0.01
LV mass index, g/m^2	48 ± 11	66 ± 15*	57 ± 10	0.001
LV mass to LV end-diastolic volume, g/ml	0.63 ± 0.13	0.95 ± 0.26*	0.90 ± 0.20*	<0.001
Peak systolic circumferential strain, negative (-) , %	18.1 ± 2.1	16.5 ± 2.6	13.4 ± 3.6†	<0.001
Peak circumferential diastolic strain rate, s^{-1}	74 ± 20	68 ± 19	56 ± 26†	0.006
Myocardial PCr/ATP ratio	2.08 ± 0.40	1.75 ± 0.29*	1.64 ± 0.32*	0.003
Myocardial triglyceride, %(Lipid/water ratio)	0.48 ± 0.28	1.14 ± 0.66*	1.22 ± 0.91*	0.004

Values are mean ±standard deviations or percentages. T2D indicates type 2 diabetes; CMR, cardiac magnetic resonance; LV, left ventricular; g, gram; g/m^2, gram per square meter; g/ml, gram per millilitre; s^{-1}, strain rate; PCr, Phosphocreatine.

*P<0.05 vs controls, with Bonferroni Correction

†P<0.05 vs lean T2D and controls, with Bonferroni Correction

Table 3: Liver Assessments

	Controls	Lean T2D patients N=15	Obese T2D patients N=27	P value
Liver Enzymes				
Bilirubin, umol/L	12 ± 4	12 ± 6	11 ± 4	0.48
ALT, IU/L	22 ± 9	30 ± 22	36 ± 17	0.18
Alk Phosphatase, IU/L	145 ± 29	150 ± 50	163 ± 46	0.47
Albumin, g/L	44 ± 3	45 ± 2	46 ± 3	0.53
Multiparametric Liver MRI				
cT1,ms	753 ± 45	821 ± 67	924 ± 116†	<0.001
Hepatic triglyceride, %(Lipid/water ratio)	3.8 ± 3.6	7.6 ± 4.6*	14.8 ± 8.4†	<0.001
T2*,ms	20 ± 4	20 ± 4	18 ± 5	0.41
Liver iron, mg/g	1.3 ± 0.12	1.34 ± 0.13	1.33 ± 0.19	0.99

Values are mean ± standard deviations or percentages. T2D indicates type 2 diabetes; umol/L, micromole per litre; IU/L, international units per litre; g/L, gram per litre; MRI, magnetic resonance imaging; cT1, corrected T1; ms, milliseconds; mg/g, milligram per gram;
*P<0.05 vs controls, with Bonferroni Correction
†P<0.05 vs lean T2D and controls, with Bonferroni Correction

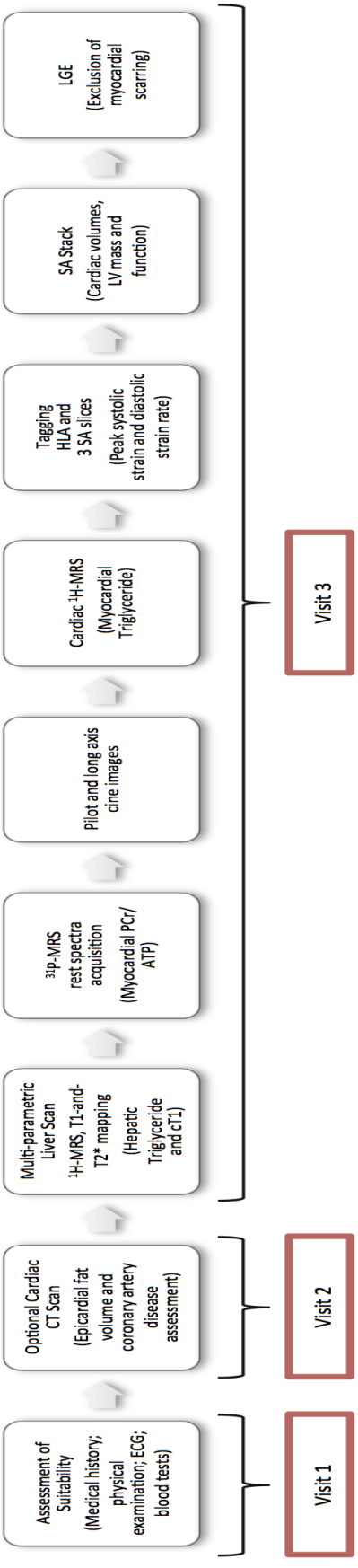


Figure 1

Study protocol for T2D patients.

Suitability of T2D patients was assessed during the first hospital visit. Those patients who consented to have a cardiac CT scan were then invited for the second hospital visit. The third hospital visit included MRI and MRS scans (3T). Multi-parametric liver MRI included ^{1}H-MRS for hepatic triglyceride; T1- and T2*-mapping yielding 'iron-corrected T1' (cT1). This was followed by CMR, which included cine imaging to assess left ventricular (LV) volumes, mass and ejection fraction; myocardial tagging for assessment of peak circumferential systolic strain and diastolic strain rate; cardiac ^{1}H-MRS for myocardial triglyceride; late gadolinium enhancement (LGE) imaging for exclusion of myocardial scarring. Controls underwent identical MRI protocols.

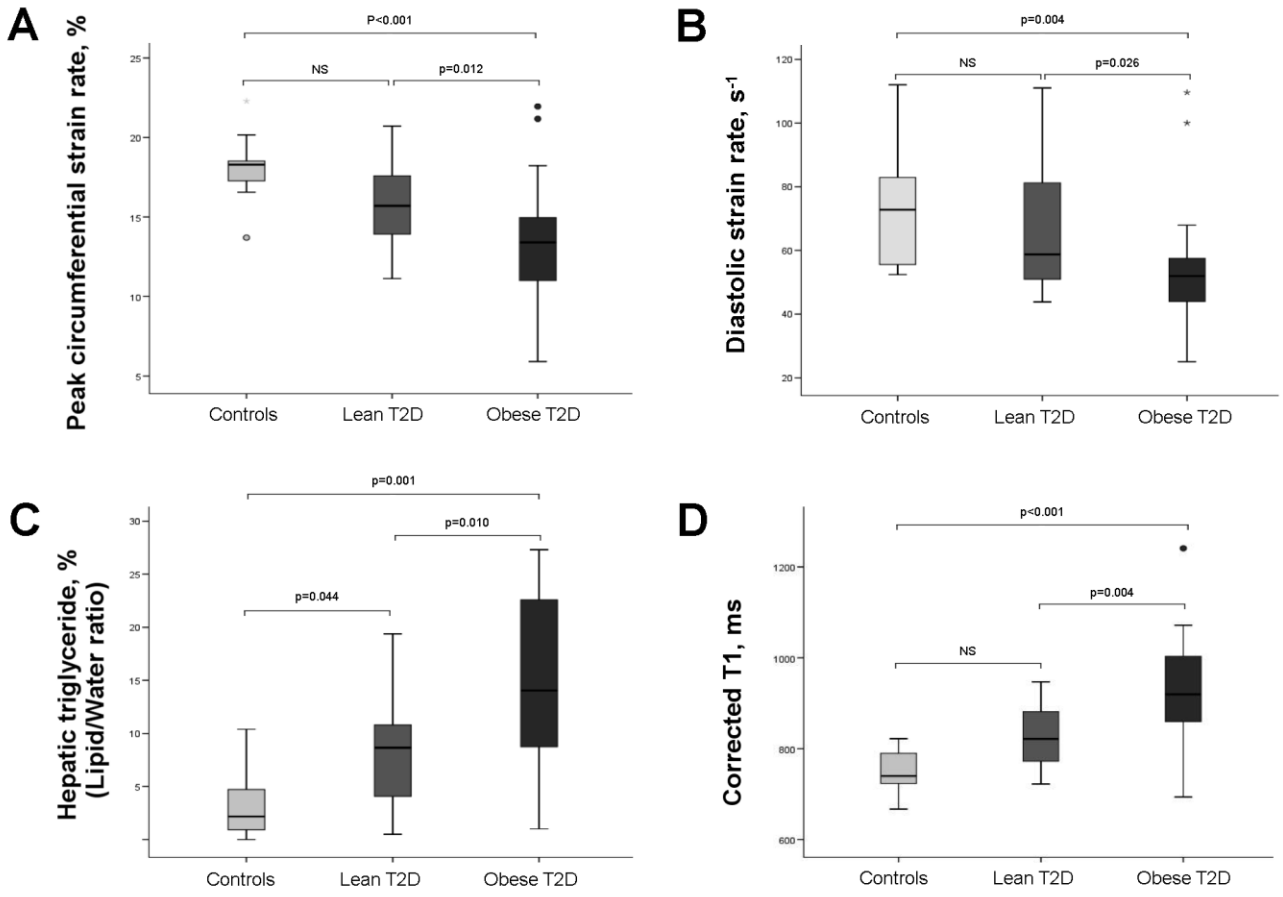
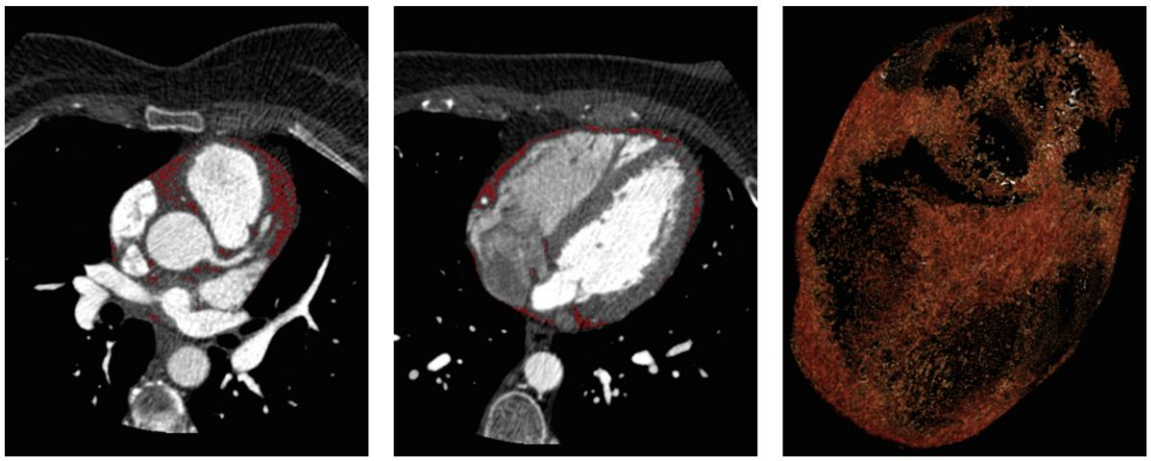
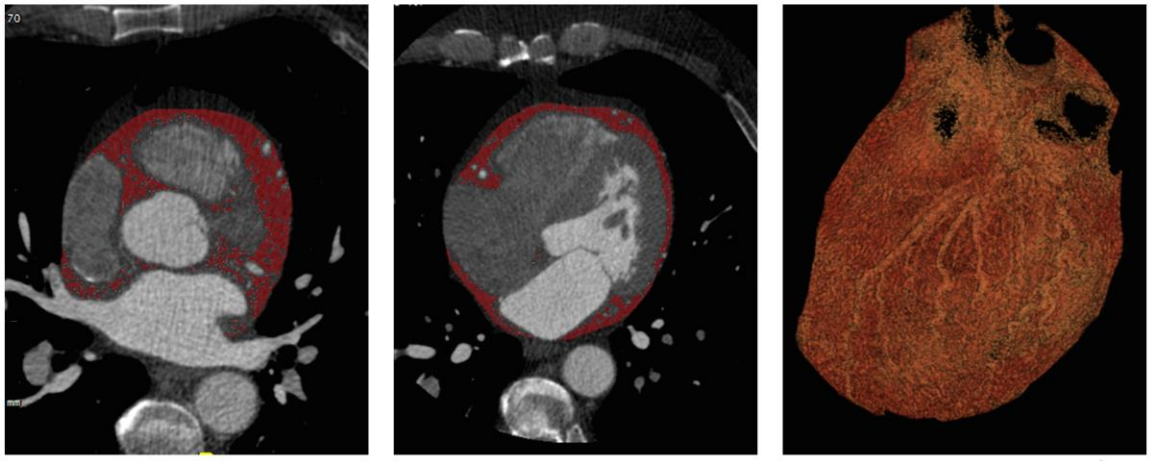


Figure 2. Differences in peak circumferential systolic strain, diastolic strain rate, hepatic steatosis, corrected T1 between lean and obese patients with T2D and healthy lean controls; (A) Peak circumferential systolic strain (B) Diastolic Strain Rate (C) Hepatic triglyceride content (%), and (D) hepatic corrected T1 map (ms)



Lean Type 2 Diabetes patient, epicardial fat volume = 37.75cm^3



Obese Type 2 Diabetes patient, epicardial fat volume = 192.59cm^3

Figure 3. Representative examples of CT epicardial fat volumes in a lean and an obese patient with T2D. Top panel: Ln-T2D patient with epicardial fat volume = 37.75cm^3; Second panel: Ob-T2D patient with epicardial fat volume = 192.59cm^3.

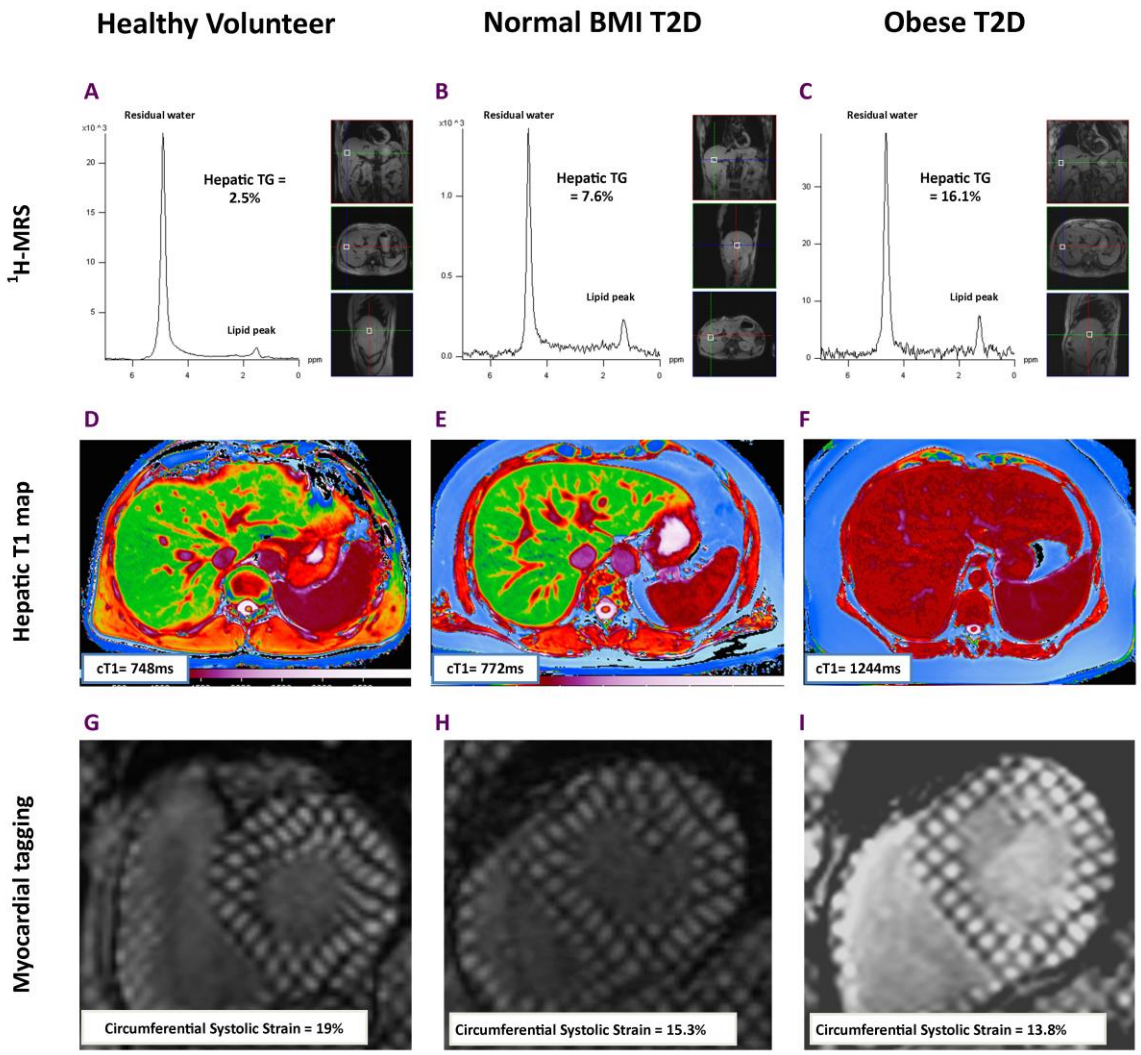


Figure 4. Representative examples of ^{1}H-MRS , trans axial liver ShMOLLI T1 map and mid-ventricular tagging in a healthy volunteer, lean and an obese patient with T2D. Top panel: A) Healthy volunteer ^{1}H-MRS with hepatic TG= 2.5%, B) Ln-T2D patient ^{1}H-MRS with hepatic TG= 7.6% , C) Ob-T2D patient hepatic TG= 16.1%; Second panel: D) Healthy volunteer with liver ShMOLLI T1 map with corrected T1= 748ms, E) Ln-T2D patient liver ShMOLLI T1 map with corrected T1= 772ms, F) Ob-T2D patient liver ShMOLLI T1 map with corrected T1= 1244ms; Third panel: G) Healthy volunteer mid-ventricular myocardial tagging with circumferential systolic strain= 19%, H) Ln-T2D patient mid-ventricular myocardial tagging

with circumferential systolic strain= 16.3%, I) Ob-T2D patient mid-ventricular myocardial tagging with circumferential systolic strain=13.8%