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Original Paper

Phenotypical heterogeneity linked to adipose tissue dysfunction in patients with Type 2 diabetes

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Abstract

Adipose tissue (AT) inflammation leads to increased free fatty acid (FFA) efflux and ectopic fat deposition, but whether AT dysfunction drives selective fat accumulation in specific sites remains unknown. The aim of the present study was to investigate the correlation between AT dysfunction, hepatic/pancreatic fat fraction (HFF, PFF) and the associated metabolic phenotype in patients with Type 2 diabetes (T2D). Sixty-five consecutive T2D patients were recruited at the Diabetes Centre of Sapienza University, Rome, Italy. The study population underwent clinical examination and blood sampling for routine biochemistry and calculation of insulin secretion [homoeostasis model assessment of insulin secretion (HOMA-β%)] and insulin-resistance [homoeostasis model assessment of insulin resistance (HOMA-IR) and adipose tissue insulin resistance (ADIPO-IR)] indexes. Subcutaneous (SAT) and visceral (VAT) AT area, HFF and PFF were determined by magnetic resonance. Some 55.4% of T2D patients had non-alcoholic fatty liver disease (NAFLD); they were significantly younger and more insulin-resistant than non-NAFLD subjects. ADIPO-IR was the main determinant of HFF independently of age, sex, HOMA-IR, VAT, SAT and predicted severe NAFLD with the area under the receiver operating characteristic curve (AUROC) = 0.796 (95% confidence interval: 0.65–0.94, $P = 0.001$). PFF was independently associated with increased total adiposity but did not correlate with AT dysfunction, insulin resistance and secretion or NAFLD. The ADIPO-IR index was capable of predicting NAFLD independently of all confounders, whereas it did not seem to be related to intrapancreatic fat deposition; unlike HFF, higher PFF was not associated with relevant alterations in the metabolic profile. In conclusion, the presence and severity of AT dysfunction may drive ectopic fat accumulation towards specific targets, such as VAT and liver, therefore evaluation of AT dysfunction may contribute to the identification of different risk profiles among T2D patients.

Key words: adipose tissue dysfunction, non-alcoholic fatty liver disease (NAFLD), pancreatic fat, type 2 diabetes (T2D).

INTRODUCTION

Adipose tissue (AT) is an endocrine organ able to influence both systemic inflammation and metabolic homoeostasis; in conditions of caloric excess it produces and releases into the bloodstream several pro-inflammatory cytokines, the 'adipokines', leading to low-grade chronic inflammation and the metabolic complications of obesity [1]. Thus, the chronic inflammatory state may provide an explanation of the well-known relationship between obesity and insulin resistance [2]. Furthermore, anatomical and functional AT rearrangements that occur in obesity, i.e. adipocyte hypertrophy and hyperplasia, lead to hypoxia, adipocyte death and attraction of active macrophages surrounding the adipocytes in crown-like structure [3]. In such a scenario, the AT

Abbreviations: ADIPO-IR, adipose tissue insulin resistance; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AT, adipose tissue; AUROC, area under the receiver operating characteristic curve; BMI, body mass index; C.I., confidence interval; CRP, C-reactive protein; DBP, diastolic blood pressure; FBG, fasting blood glucose; FBI, fasting blood insulin; FFA, free fatty acid; FLI, fatty liver index; GRE, gradient echo; HbA_{1c}, glycosylated haemoglobin; HDL-C, high-density lipoprotein cholesterol; HFF, hepatic fat fraction; HOMA-IR, homoeostasis model assessment of insulin resistance; HOMA-β%, homoeostasis model assessment of insulin secretion; IP, in-phase; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OP, opposed-phase; PFF, pancreatic fat fraction; ROI, region of interest; SAT, subcutaneous adipose tissue; SBP, systolic blood pressure; T2D, Type 2 diabetes; VAT, visceral adipose tissue; γ-GT, γ-glutamyl transpeptidase.

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insulin resistance and its capability of storing triacylglycerols is drastically reduced, along with an increased lipolytic activity. This promotes a hyper-afflux of free fatty acids (FFAs) into the bloodstream and aberrant fat deposition in non-ATs [1]. An excessive intrahepatic fat accumulation is the hallmark of non-alcoholic fatty liver disease (NAFLD), a condition potentially evolving into non-alcoholic steatohepatitis (NASH), cirrhosis, liver failure and hepatocarcinoma [4,5]. NAFLD represents nowadays the most common liver disorder in the Western world [6] and is an emerging independent risk factor for cardiovascular disease [7]. A strong association between NAFLD and Type 2 diabetes (T2D) has been demonstrated: more than 90% of obese patients with T2D have NAFLD, and the link between these two universally widespread conditions is represented by insulin resistance [8]. Furthermore, lipid deposition in the hepatocytes leads to a critical reduction in hepatic insulin sensitivity, resulting in a deregulation of glucose/insulin homoeostasis, systemic insulin resistance and increased fasting glucose levels [9–12]. More recently, the intrapancreatic fat accumulation has been suggested as a pathological condition itself, with an increased prevalence in obese subjects and in individuals with the metabolic syndrome and diabetes [13,14]. Some studies investigated the impact of pancreatic fat content on β-cell function in both healthy people and subjects with impaired glucose regulation, but evidence is contrasting [15–20]. In children, fatty pancreas seems to represent a hallmark of metabolic impairment [21]; however, its role in insulin secretion and metabolic control in T2D patients is still unknown and, very recently, even the existence itself of an intrapancreatic – and not just peripancreatic – fat accumulation, has been questioned [20,22,23]. Furthermore, to the best of our knowledge, no study has investigated whether fat deposition into the pancreas is mediated by AT dysfunction, as in NAFLD, and whether fatty pancreas could represent an additional marker of AT inflammation and metabolic impairment in patients with T2D.

Therefore, the aim of the present study was to study the association between the presence and severity of AT dysfunction, quantified by the histologically validated adipose tissue insulin resistance (ADIPO-IR) index, and fat accumulation both in liver and pancreas in an adult population of T2D patients. We also tested the hypothesis of an influence of AT dysfunction in orienting fat localization towards specific targets, such as subcutaneous (SAT) or visceral (VAT) AT compartments. Finally, we sought a different role of hepatic fat fraction (HFF) and pancreatic fat fraction (PFF) in identifying different risk profiles among diabetes patients.

MATERIALS AND METHODS

Study population

For this purpose, we analysed the cross-sectional data derived from the population of the Eudract 2011-003010-17 study (European Union Clinical Trials Register, http://www.clinicaltrialsregister.eu), a randomized controlled trial aiming to evaluate the efficacy and safety of oral vitamin D supplementation in T2D patients with NAFLD [24]. The study protocol was reviewed and approved by the Ethics Committee of this hospital and the study was conducted in conformance with the Helsinki Declaration. Written informed consent was obtained from all patients before the study.

Sixty-seven patients affected by T2D and with clinical suspicion of NAFLD [increased serum transaminases levels, low aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio and/or ultrasound-detected fatty liver in the absence of known hepatic chronic disease] were selected among those referring to our Diabetes Outpatients Clinics, Sapienza University of Rome, Rome, Italy, for routine diabetes care. Two subjects withdrew their consent to study participation before undergoing abdominal magnetic resonance (MRI).

To be eligible for the study, patients had to fulfil the following criteria: male or female subjects with a diagnosis of T2D, 25–70 years of age, no history of current or past excessive alcohol drinking, as defined by an average daily consumption of alcohol <30 g/day in men and <20 g/day in women, negative tests for the presence of hepatitis B surface antigen and antibody against hepatitis C virus, absence of history and findings consistent with cirrhosis and other chronic liver diseases.

All subjects underwent a complete work-up including a clinical examination, anthropometric measurements and laboratory tests. Weight and height were measured with patients wearing light clothing and no shoes. The body mass index (BMI) was calculated as weight in kg divided by the square of the height in m (kg/m^2). Waist circumference (cm) was measured midway between the 12th rib and the iliac crest. Systemic blood pressure (systolic, SBP; diastolic, DBP; mmHg) was measured after 5 min of rest using an electronic auscultatory blood pressure recorder with an appropriately sized cuff based on the measurement of arm circumference with the patient sitting in the upright position. Three measurements were recorded, and the average of the second and third measurement was recorded and used in the analyses.

Abdominal MRI was performed to assess HFF and PFF (%) and to measure SAT and VAT (cm^2).

Laboratory determinations

The study population underwent fasting blood sampling to assess blood glucose (FBG, mg/dl), glycosylated haemoglobin (HbA_{1c}, %, mmol/l), total cholesterol (mg/dl), high-density lipoprotein cholesterol (HDL-C, mg/dl), triacylglycerols (mg/dl), AST (IU/l), ALT (IU/l), γ-glutamyl transpeptidase (γ-GT, IU/l) and C-reactive protein (CRP, mg/dl) by standard laboratory methods. Serum insulin [fasting blood insulin (FBI), μ-units/l] was measured by radio immuno assay (Pantec; intra- and inter-assay coefficients of variation <5%), FFAs, (mg/dl) by colorimetric methods (Bios) and circulating adiponectin levels (μg/ml) were assessed by enzyme-linked immunosorbent assay (ELISA) (Tema Ricerca; intra- and inter-assay coefficients of variation $\leqslant 5$%).

The low-density lipoprotein cholesterol (LDL-C, mg/dl) value was calculated using the Friedwald formula. The homoeostasis model assessment of insulin resistance (HOMA-IR) and insulin secretion (HOMA-β%) were calculated as previously described [25], the AT insulin resistance was quantified by the ADIPO-IR index [FFAs (μmol/l) $\times$ FBI (μU/l)] [26–28]. The fatty liver index (FLI), a clinical correlate of NAFLD, was also

calculated in the whole study population [29]. Diabetes mellitus was diagnosed according to American Diabetes Association (ADA) 2009 criteria [30].

MRI evaluations

All MRI evaluations were performed by the same operator, unaware of the study aims and blinded to laboratory values, using a 1.5-T magnet (Magnetom Avanto, Siemens Medical Systems) equipped with a phased-array surface coil and a spine array coil. Image acquisition was performed in the axial plane during an end-expiratory breath-hold using a sensitivity encoding (SENSE) technique in order to reduce the overall acquisition time to approximately 15 s. HFF and PFF were obtained by using a 2D spoiled gradient echo (GRE) acquired on the axial plane. To minimize T1 effects, a low flip angle (10°) was used at a repetition time of 150 ms. To estimate fat-water signal interference and T2* effects, three echoes were obtained at serial opposed-phase (OP) and in-phase (IP) echo times (2.3, 4.7 and 6.9 ms); other parameters applied were: section thickness, 5 mm; matrix size, 256 × 182; field of view, 35 cm × 40 cm. HFF and PFF were calculated from the mean of the two IP sequences (IP correct) with the OP sequence subtracted and then divided by the '2× IP correct' sequence. For HFF, eight different regions of interest (ROIs) measuring 2 cm^2 were drawn, one for each hepatic segment within the liver, avoiding areas with vessels, motion artefacts and partial volume effects; ROIs were placed at anatomically matched locations on paired images by using a co-registration tool available on the picture archiving and communication system workstation. Finally, mean ± S.D. HFF was calculated for each patient and NAFLD was diagnosed in the presence of mean HFF $\geqslant$ 5.5% [31]. For PFF, three ROIs of 0.5 cm^2 were placed respectively in the pancreas's head, body and tail, in areas with homogeneous fat distribution and far from the pancreas borders, in order to avoid bias related to the presence of peripancreatic visceral fat.

For VAT and SAT quantification, a 3D GRE T1-weighted volume-interpolated breath-hold examination (VIBE) sequence on axial plane modified by Dixon was acquired [repetition time (TR), 4.7 ms; echo time (TE), 2.3 ms; flip-angle, 10°C; matrix, 256 mm×192 mm; section thickness, 5 mm, reconstructed 2.5 mm; intersection gap, 0]. The fat-only datasets were transferred to a personal computer for analysis using commercially available software (Slice-O-Matic; Tomovision); the detailed procedures are described elsewhere [31–33]. Briefly, data were calculated from AT area at L1–L2, L2–L3, L3–L4 and L4–L5 levels; a free-form ROI and manual threshold were used to select fat tissue within VAT and SAT slides. Mean ± S.D. VAT and SAT areas were then calculated in each patient for statistical purposes.

Statistics

SPSS version 23 statistical package was used to perform all the analyses. Values are reported as means ± S.D. for continuous variables and as a percentage for categorical variables. Comparisons between two groups were performed by the non-parametric Kruskal–Wallis test for continuous variables and Pearson's χ^2 test for categorical variables. Bivariate correlation analyses were calculated by Spearman's rank correlation or by age–sex-adjusted partial correlation test; the multivariate regression model included sex and age and all the variables were statistically significant at the bivariate correlation analysis. A two-tailed P value < 0.05 was considered statistically significant, with a confidence interval (C.I.) of 95%.

RESULTS

AT insulin resistance and NAFLD

The prevalence of NAFLD in our study population was 55.4% ($n = 36/65$); T2D patients with NAFLD were significantly younger (56.2 ± 9.7 compared with 61.7 ± 8.8 years, $P = 0.01$) despite comparable diabetes duration, and more insulin resistant (HOMA-IR: 14 ± 5.1 compared with 10.2 ± 5.3, $P = 0.004$, ADIPO-IR: 7.3 ± 3.9 compared with 5 ± 4.6, $P = 0.008$) than subjects without NAFLD.

Blood lipids and glycaemic control, as expressed by FBG, HbA_{1c} and diabetes treatment (type and number of oral antidiabetic agents, insulin therapy) were comparable between the two subgroups. T2D patients with NAFLD had double serum CRP levels than non-NAFLD subjects. Clinical and biochemical parameters of T2D patients according to the presence of NAFLD are shown in Table 1. The differences found in insulin-derived indexes between T2D individuals with and without NAFLD persisted after the exclusion of insulin-treated patients ($n = 11$) from the whole study population (results not shown). T2D patients with NAFLD had comparable mean PFF, VAT and SAT with those without NAFLD (PFF: 5.1 ± 5% compared with 5.4 ± 5.3%, $P = 0.8$; VAT: 195.2 ± 74.6 cm^2 compared with 184.5 ± 69.3 cm^2, $P = 0.56$; SAT: 255.1 ± 130.1 cm^2 compared with 219.6± 108.8 cm^2, $P = 0.23$).

The bivariate correlation analysis showed that an increased HFF, considered as a continuous variable, was associated with higher serum transaminases and FBI, low AST/ALT ratio and greater AT dysfunction and insulin resistance (Table 2); notably, multivariate logistic regression analysis demonstrated that greater ADIPO-IR was a determinant of increased HFF independent of sex, age and all the metabolic confounders (standardized β: 0.41, $P = 0.012$). The area under the receiver operating characteristic curve (AUROC) of ADIPO-IR for predicting the presence of severe NAFLD, considered as belonging to the highest quartile of HFF, was 0.796 (C.I. 95%: 0.65–0.94, $P < 0.001$); an ADIPO-IR greater than 6.9 mmol/l·μ-units/ml was able to predict severe NAFLD with a sensitivity of 84.6% and a specificity of 79.1% (Figure 1).

In the whole study population, higher ADIPO-IR correlated with the presence of obesity, atherogenic dyslipidaemia, increased serum transaminases and CRP levels, FLI, HFF, VAT and whole-body insulin resistance, but was not associated with sex, age and T2D duration (Table 3). Fitting into the highest ADIPO-IR quartile (worse AT insulin resistance) significantly predicted the presence of NAFLD in T2D patients, with odds ratio (OR): 3.3 (C.I. 95%: 1.1–9.8, $P = 0.032$, χ^2 test).

AT insulin resistance and PFF

Our study population had a mean ± S.D. PFF of 5.2 ± 5%, significantly higher than that reported in a historical control

Table 1 Clinical and biochemical characteristics of T2D patients with and without NAFLD (cut-off HF content: 5.5% by MRI).
n.s., not significant. Mann–Whitney test, except *χ^2 test, †ANOVA test.

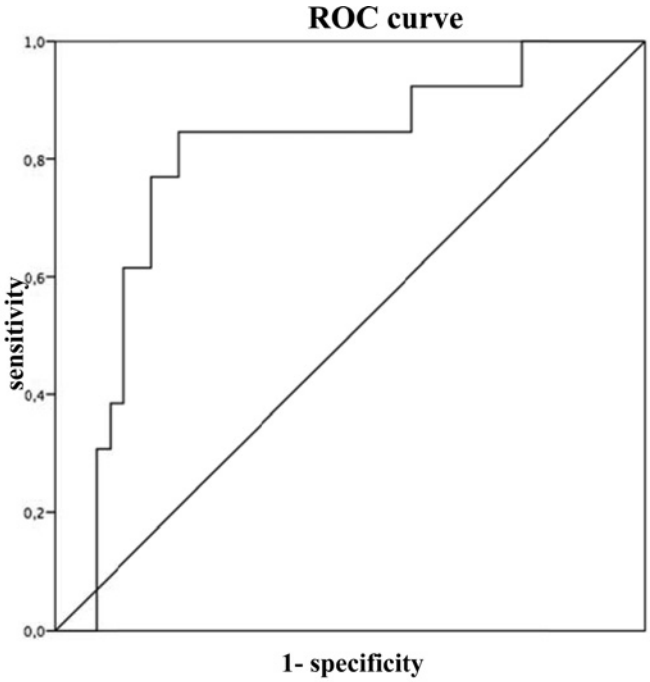
	NAFLD ($n = 36$)	Non-NAFLD ($n = 29$)	*P*-value
Age (years)	**56.2 ± 9.7**	**61.7 ± 8.8**	**0.010**
Sex (males/females)	26/10	21/8	n.s.*
BMI (kg/m^2)	30.4 ± 4.4	29.4 ± 4.2	n.s.
Waist circumference (cm)	106.2 ± 14.2	100.4 ± 10.2	n.s.
T2D duration (years)	6 ± 5	8.3 ± 8	n.s.
SBP (mmHg)	127.5 ± 16.3	133.9 ± 16.6	n.s.
DBP (mmHg)	81 ± 9.6	81.8 ± 10.4	n.s.
Total cholesterol (mg/dl)	176.2 ± 36.9	176.4 ± 38.1	n.s.
HDL-C (mg/dl)	48.4 ± 15.1	49.9 ± 12.9	n.s.
LDL-C (mg/dl)	100.7 ± 35.1	98.9 ± 31.4	n.s.
Triacylglycerols (mg/dl)	135 ± 65.8	137.3 ± 59	n.s.
FBG (mg/dl)	130.4 ± 32.3	134.8 ± 46	n.s.
HbA_{1c} (%/mmol/mol)	6.7 ± 1/50 ± 10	6.5 ± 0.9/48 ± 8	n.s.
AST (IU/l)	26.9 ± 13.3	20.8 ± 11.5	0.012
ALT (IU/l)	39.6 ± 25.2	24.3 ± 12.1	0.001
γ-GT (IU/l)	51.2 ± 61.7	32.3 ± 33.2	n.s.
AST/ALT	0.74 ± 0.2	0.92 ± 0.3	0.005
FFAs (μmol/l)	549.2 ± 281	484.8 ± 215.5	n.s.
FBI (μ-units/l)	14 ± 5.1	10.2 ± 5.3	0.004
FLI	70.2 ± 23.3	59.3 ± 26.1	n.s.
HOMA-IR	4.4 ± 1.6	3.2 ± 1.9	0.025
HOMA-β%	103.3 ± 72.2	70.8 ± 48.8	n.s.
Quantitative insulin sensitivity check index (QUICKI)	0.31 ± 0.02	0.33 ± 0.03	0.025
ADIPO-IR	7.3 ± 3.9	5 ± 4.6	0.008
CRP	4.2 ± 5	2.1 ± 2.7	0.05
Adiponectin (μg/ml)	6.5 ± 3	6.1 ± 3.8	n.s.
Insulin treatment (*n* patients/%)	4/9	7/28	n.s.*
Number of oral anti-diabetic agents (% patients)			
0	12	15	n.s.†
1	47	43	
2	32	27	
3	9	15	
Statin treatment (*n* patients/%)	18/52	18/64	n.s.*
Anti-hypertensive treatment (*n* patients/%)	29/80	21/72	n.s.*

cohort of sex- and age-matched healthy controls recruited in our centre ($n = 30$, males/females: 21/9, age: 55.1 ± 7.9 years; PFF: 3 ± 2%, $P < 0.05$). Patients with T2D within the highest PFF quartile were significantly older and had increased BMI, waist circumference, SBP and serum CRP levels in comparison with those in the lowest PFF quartile (Table 4). Moreover, although patients with the highest PFF showed a trend to worse glycaemic control despite a higher rate of insulin-treated subjects, the positive association between PFF and HbA_{1c} disappeared after removing the effect of sex and age (partial bivariate correlation: $r = 0.23$, $P = 0.065$).

Whereas PFF was significantly associated with age ($r = 0.30$, $P = 0.01$), T2D duration ($r = 0.37$, $P = 0.002$), BMI ($r = 0.31$, $P = 0.012$), waist circumference ($r = 0.30$, $P = 0.016$), and both VAT and SAT area ($r = 0.29$, $P = 0.02$; $r = 0.37$, $P = 0.002$), no correlation was found between increased PFF and AT dysfunction (ADIPO-IR: $r = 0.09$, $P = 0.50$), HOMA-IR ($r = 0.02$, $P = 0.85$) and HOMA-β% ($r = 0.01$, $P = 0.92$) at the bivariate correlation analyses.

Interestingly, a higher PFF was not associated with the diagnosis of NAFLD ($r = -0.09$, $P = 0.43$), or with HFF ($r = -0.11$, $P = 0.38$) and serum transaminases (AST: $r = -0.09$, $P = 0.48$; ALT: $r = -0.21$, $P = 0.09$; γ-GT: $r = -0.16$, $P = 0.21$); the partial correlation analysis confirmed the lack of an association between PFF and HFF even after removing the sex/age effect ($r = -0.075$, $P = 0.56$).

Finally, an increased adiposity, as expressed by BMI, and not the AT insulin resistance, strongly predicted higher PFF independently of all clinical and metabolic confounders ($R^2 = 0.32$, $P = 0.012$; Table 5).



Area under the curve

Area	Standard error[a]	Asintotic significance[b]	Asintotic 95%Confidence Interval	
			Lower limit	Upper limit
0.796	0.073	0.001	0.653	0.939

a. Based on non-parametric assumption

b. null hypothesis: real area = 0.5

Figure 1 ADIPO-IR AUROC for severe NAFLD

AT insulin resistance, VAT and SAT area

Mean VAT area was significantly higher in T2D males than in females (202.8 ± 74.2 cm^2 compared with 158.4 ± 55.7 cm^2, $P = 0.013$) as opposed to mean SAT area which was greater in female than male participants (314.5 ± 103.2 cm^2 compared with 210.4 ± 116.3 cm^2, $P = 0.001$).

Age and sex-adjusted partial bivariate analyses showed the existence of a significant association between increased VAT area and higher ADIPO-IR ($r = 0.28$, $P = 0.035$), SBP ($r = 0.32$, $P = 0.029$), FBI and HOMA-IR ($r = 0.37$, $P = 0.024$; $r = 0.40$, $P = 0.005$), FLI ($r = 0.6$, $P < 0.001$), CRP ($r = 0.48$, $P < 0.001$) and low HDL-C ($r = -0.29$, $P = 0.04$); higher VAT area also correlated with higher PFF ($r = 0.45$, $P < 0.001$) but not with higher HFF ($r = 0.18$, $P = 0.21$), serum adiponectin ($r = -0.16$, $P = 0.21$) and FFA concentration ($r = 0.05$, $P = 0.71$).

As for mean SAT area, no association was found with ADIPO-IR ($r = 0.06$, $P = 0.7$) and all other clinical and biochemical markers of metabolic disease, although increased SAT area significantly correlated with higher CRP levels ($r = 0.36$, $P = 0.01$) and PFF ($r = 0.45$, $P < 0.001$).

AT insulin resistance and systemic inflammation

In T2D patients, increased serum CRP levels were associated with greater ADIPO-IR ($r = 0.35$, $P = 0.008$), BMI ($r = 0.44$, $P < 0.001$), waist circumference ($r = 0.38$, $P = 0.003$), SAT ($r = 0.50$, $P < 0.001$), DBP ($r = 0.26$, $P = 0.04$) and FLI ($r = 0.46$, $P < 0.001$).

In order to identify clinical and metabolic correlates of increased CRP levels in T2D non-obese patients, we excluded from the analysis all subjects with a BMI > 29.9 kg/m^2 and found that in the non-obese subgroup ($n = 31$ patients, 48%) higher CRP concentrations characterized T2D patients with NAFLD + worse AT dysfunction (as expressed by ADIPO-IR above the median value), compared with those with NAFLD but low ADIPO-IR, and were associated with worse liver inflammation (AST $P < 0.001$, ALT $P = 0.01$, γ-GT $P = 0.02$).

Table 2 HFF % – bivariate correlation analyses (Spearman's coefficient, continuous variable)

	Correlation coefficient	*P*-value
HOMA-IR	0.32	0.015
ADIPO-IR	0.41	0.002
Age	−0.29	0.019
AST	0.49	<0.001
ALT	0.57	<0.001
γ-GT	0.38	0.002
FBI	0.36	0.006
Quantitative insulin sensitivity check index (QUICKI)	−0.32	0.015
AST/ALT	−0.41	<0.001

Table 3 ADIPO-IR – bivariate correlation analyses (Spearman's coefficient, continuous variable)
n.s., not significant.

	Correlation coefficient	*P*-value
Age	−0.11	n.s.
Sex (M/F)	0.20	n.s.
T2D duration	−0.10	n.s.
BMI	−0.31	0.019
PAD	0.45	<0.001
HDL-C	−0.29	0.03
Triacylglycerols	0.36	0.006
AST	0.27	0.04
ALT	0.32	0.01
γ-GT	0.28	0.04
CRP	0.35	0.008
HOMA-IR	0.58	<0.001
HOMA-β%	0.30	0.025
Quantitative insulin sensitivity check index (QUICKI)	−0.58	<0.001
FLI	0.40	0.003
HFF	0.41	0.002
NAFLD (yes/no) (NMR)	0.36	0.007
Obesity (yes/no)	0.31	0.02
VAT	0.28	0.035
SAT	0.22	0.10

DISCUSSION

In the present study we demonstrated that the occurrence of NAFLD in patients with T2D is linked to the presence of AT dysfunction whereas fat accumulation into the pancreas does not appear to be associated with AT dysfunction and insulin-resistance.

Our results, indeed, point towards the existence of a marked heterogeneity in intraparenchymal fat accumulation among patients with T2D, with a pattern of distribution which appears to be related more to AT dysfunction than to adiposity itself.

As far as we are aware, this is the first study investigating the association between AT dysfunction and intrapancreatic fat accumulation, even in relation to the presence of NAFLD.

Over half of our population of T2D patients had MRI-detected fatty liver; NAFLD was more prevalent in younger subjects with worse insulin resistance and AT dysfunction, regardless of total adiposity, diabetes duration, therapy and metabolic control. A number of studies found a correlation between HFF and visceral adiposity in the general population and between liver inflammation and fibrosis and VAT area among subjects with biopsy-proven NASH [31,34–36]. Our study demonstrated that in T2D patients, a population with increased VAT area and aberrant VAT/SAT ratio by definition, the main determinant of high HFF is AT dysfunction, and then AT resistance to insulin action, independent of BMI and total adiposity. Moreover, we were able to identify a cut-off value of ADIPO-IR predictive of the presence of severe NAFLD in T2D patients, therefore allowing us to stratify the risk of NASH and, subsequently, liver-related complications [4,5] and cardiovascular disease [8] in this population.

The ADIPO-IR threshold we detected is in line with the mean values reported in obese patients with NAFLD, which is 4-fold higher than that of healthy obese subjects or lean individuals without NAFLD and T2D [28]. In our population, we identified a remarkable heterogeneity of AT dysfunction severity, mostly related to greater insulin resistance, higher HFF, serum liver enzymes, CRP, presence of atherogenic dyslipidaemia and increased fat storage into the VAT – but not SAT – compartment. In agreement with our results, du Plessis et al. [37] recently demonstrated the association between NAFLD/NASH and the expression of pro-inflammatory cytokines in AT biopsies of morbidly obese subjects. As AT remodelling leads to increased FFA efflux into the bloodstream and fat accumulation into the liver, we investigated whether AT dysfunction could determine aberrant fat storage in pancreatic parenchyma as well, and, thus, whether pancreatic steatosis could represent an additional marker of AT inflammation and metabolic impairment in patients with T2D. Indeed, the main determinant of increased intrapancreatic fat accumulation was total adiposity, in terms of higher BMI, SAT and VAT areas, whereas no correlation was found between PFF and AT dysfunction, systemic insulin resistance and insulin secretion. Ultrasound-detected fatty pancreas has been associated with metabolic syndrome and its components in cohorts of consecutive subjects and in some case–control studies [13,14,38]. Contrariwise, the pancreatic parenchymal fat distribution within a population of T2D patients was assessed by MRI and quantified by specific software, thus excluding peripancreatic fat from the mean PFF. In agreement with Wong et al. [19], reporting an association between MRI-detected PFF and central adiposity, but not insulin secretion, in a large cohort of healthy Chinese volunteers, we did not find a correlation between PFF and either insulin secretion or glycaemic control. So far, evidence on pancreatic fat and its correlates in T2D patients is limited; although most studies demonstrated an association between increased pancreatic fat content, age and total adiposity, data on PFF and insulin secretion, in the presence of impaired glucose regulation or diabetes, are contrasting and far from being conclusive [15–18,20–23,39]. Interestingly, we did not find any association between the

Table 4 Clinical and biochemical characteristics of T2D patients according to PFF (I compared with IV quartile)
n.s., not significant. Mann–Whitney test, except *χ^2 test, †ANOVA test.

	Low PFF	High PFF	*P*-value
Pancreatic fat content (%)	0 ± 2	12.9 ± 5	**<0.001**
Age (years)	53.9 ± 11.7	63.4 ± 8.8	**0.015**
Sex (males/females)	15/1	10/6	**0.03***
BMI (kg/m^2)	27.7 ± 2.6	32.4 ± 5.4	**0.005**
Waist circumference (cm)	97.8 ± 5.4	109.6 ± 15.1	**0.007**
T2D duration (years)	4.5 ± 5	8.2 ± 7.9	n.s.
SBP (mmHg)	121.6 ± 11.8	139.2 ± 22	**0.008**
DBP (mmHg)	80 ± 7.3	83.9 ± 12.8	n.s.
Total cholesterol (mg/dl)	175.13 ± 44.3	179.1 ± 44.7	n.s.
HDL-C (mg/dl)	45.4 ± 10	52.3 ± 16.2	n.s.
LDL-C (mg/dl)	100 ± 37.3	99.1 ± 40.7	n.s.
Triacylglycerols (mg/dl)	148.2 ± 90.1	137.4 ± 56	n.s.
FBG (mg/dl)	124.2 ± 27.9	132.1 ± 41.2	n.s.
HbA_{1c} (%/mmol/mol)	6.17 ± 0.7/44 ± 7	6.7 ± 0.9/50 ± 8	**0.05**
AST (IU/l)	22.6 ± 7.2	20.01 ± 5.7	n.s.
ALT (IU/l)	34.9 ± 18	24.8 ± 12.4	n.s.
γ-GT (IU/l)	34.3 ± 22.9	37.1 ± 57.8	n.s.
AST/ALT	0.7 ± 0.2	0.9 ± 0.3	**0.03**
FFAs (μmol/l)	484.9 ± 355.7	481.6 ± 181.4	n.s.
FBI (μ-unit/l)	12.9 ± 3.9	12.2 ± 4.3	n.s.
FLI	57.2 ± 19.3	71.7 ± 23.2	n.s.
HOMA-IR	3.8 ± 1.2	4 ± 1.8	n.s.
HOMA-β%	97 ± 71.4	82.2 ± 44.3	n.s.
Quantitative insulin sensitivity check index (QUICKI)	0.32 ± 0.02	0.32 ± 0.04	n.s.
ADIPO-IR	5.8 ± 4.6	5.8 ± 2.8	n.s.
CRP	1.2 ± 2	4.2 ± 4.5	**0.03**
Adiponectin (μg/ml)	6.3 ± 3.4	5.6 ± 3	n.s.
Insulin treatment (*n* patients/%)	0/0	3/31	**0.02***
Number of oral anti-diabetic agents (% patients)			
0	26.7	6.2	n.s.†
1	46.7	50	
2	20	31.2	
3	6.6	12.6	
Statin treatment (*n* patients/%)	6/40	9/56	n.s.*
Anti-hypertensive treatment (*n* patients/%)	11/69	12/75	n.s.*

Table 5 Multivariate linear regression analysis
Dependent variable: PFF (%).

	B	S.D.	β	*t*	*P*-value
(Constant)	−0.185	0.084		−2.197	0.033
Age	0.001	0.001	0.116	0.879	0.384
Sex	0.025	0.017	0.193	1.458	0.152
BMI	0.005	0.002	0.379	2.622	0.012
ADIPO-IR	0.000	0.002	−0.020	−0.153	0.879
HbA_{1c}	0.002	0.009	0.031	0.206	0.838
Insulin therapy	0.021	0.022	0.145	0.972	0.336
CRP	0.001	0.002	0.048	0.360	0.720
AST/ALT	0.029	0.027	0.138	1.067	0.291

diagnosis of NAFLD and high PFF in T2D, differently from what reported in healthy subjects [19,40] and in obese non diabetic individuals [41,42]. Therefore, in our study, specific parenchymal fat distribution identified different phenotypes of T2D patients: subjects with NAFLD were younger and had worse metabolic profile than non-NAFLD subjects, whereas higher PFF identified older patients with AT dysfunction and insulin secretion comparable with those found in T2D subjects with lower PFF.

Since adipocytes from VAT are more prone to lipolysis than subcutaneous adipocytes [43,44], FFAs may flood directly from VAT into the portal vein, exposing the liver to higher FFA levels than those predictable from systemic FFA levels [45–47]. In an elegant experiment of lipid physiology, Jensen et al. [47] tested a model to predict the fraction of hepatic FFA delivery that arises from VAT lipolysis. For this purpose, splanchnic palmitate kinetics were measured in blood samples collected from the arterial, portal venous and hepatic venous circulation of chronically catheterized dogs under several experimental conditions. The overall results showed that liver took up a large proportion of FFAs entering the splanchnic bed through the portal vein, with only a small fraction of FFAs being taken up by non-hepatic splanchnic blood [47]. Furthermore, human studies showed that, in conditions of hyperinsulinaemia, the circulating amount of FFAs derived from VAT increases drastically, thus exposing the liver to significantly greater FFA concentrations than the periphery. This portal–systemic difference may be even higher in condition of visceral obesity [48]. Indeed, we may speculate that AT dysfunction results in greater FFA flux into the portal system, leading to hepatic, but not pancreatic, aberrant fat accumulation. These data, along with our findings of comparable BMI, waist circumference, VAT and SAT areas between NAFLD and non-NAFLD diabetic patients, reinforce the assumption for a key role of AT dysfunction in determining NAFLD in this population. Conversely, evidence showed that greater waist circumference and increased abdominal fat depend on both SAT and VAT expansion in the presence of inflamed and dysfunctional AT [49], arising doubts on the exclusive role of VAT mass in causing ectopic fat deposition and its complications [50,51]. Another explanation of our findings comes from the striking evidence that NAFLD itself is a determinant of insulin resistance [9–12] and impaired insulin secretion [52], and represents an additional risk factor for metabolic complications [53]. Our findings corroborate in a clinical setting the bulk of the experimental data on a direct role of fatty liver in driving and exacerbating the progression of dysmetabolic conditions. In the presence of AT dysfunction, once NAFLD is established, metabolic pathways triggered by the intrahepatic fat accumulation may directly induce detrimental outcomes in T2D patients, whereas other processes, such as intrapancreatic fat accumulation, probably represent a consequence of an overall increased adiposity in this population.

Despite the cross-sectional design of the present study, not allowing us to establish a certain causal relationship, we may speculate that intrapancreatic fat does not represent, itself, a risk factor and/or a marker of further metabolic impairment in patients with established diabetes, and, unlike NAFLD, is not directly related to AT inflammation.

In conclusion, our findings suggest that the presence and severity of AT dysfunction may determine ectopic fat distribution towards specific targets, such as VAT and liver, thus identifying different risk profiles in T2D patients and potentially representing a starting point for future research aimed at identifying novel therapeutic approaches.

AUTHOR CONTRIBUTION

IB, MGC, FA, SM, RDV and AF designed the study. IB, MGC, FAC and LB coordinated the study, oversaw patient recruitment and finalized the dataset. MDB, LP, FAC and LB performed clinical evaluations, oversaw collection and analysis of biological samples. IB, MGC and MGB conducted the statistical analyses. MDM and CC performed the MRI and analysed the dataset. IB and MGC drafted the paper, which was reviewed by all authors. All authors read and approved the final manuscript.

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