Atherosclerosis 288 (2019) 51–59

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 and angiopoietin-like protein 4 are associated with the increase of lipoprotein lipase activity in epicardial adipose tissue from diabetic patients

Magalí Barchuk[a], Laura Schreier[a], Graciela López[a], Agata Cevey[b], Julio Baldi[c], María del Carmen Fernandez Tomé[d], Nora Goren[b], Miguel Rubio[c], Verónica Miksztowicz[a,e], Gabriela Berg[a,e,*]

[a] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC), Departamento de Bioquímica Clínica, Laboratorio de Lípidos y Aterosclerosis, Buenos Aires, Argentina
[b] Universidad de Buenos Aires, CONICET, Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Facultad de Medicina, Buenos Aires, Argentina
[c] Universidad de Buenos Aires, Hospital de Clínicas "José de San Martín", División de Cirugía Cardíaca, Buenos Aires, Argentina
[d] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Biología Celular y Molecular, Instituto de Química y Fisicoquímica Biológicas (IQUIFIB), CONICET, Buenos Aires, Argentina
[e] Universidad de Buenos Aires, CONICET, Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina

HIGHLIGHTS

- LPL activity was increased in EAT from DM2 patients.
- In DM2, increased GPIHBP1 and decreased ANGPTL4 EAT expression modulated LPL activity.
- Higher EAT LPL activity was responsible for TG-VLDL catabolism and fatty acids release.
- EAT VLDL receptor was inversely associated with circulating VLDL mass and TG-VLDL.
- The higher EAT LPL activity in DM2 could be responsible for the increased EAT volume.

ARTICLE INFO

Keywords:
Epicardial adipose tissue
Lipoprotein lipase
Glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1
Angiopoietin-like protein 4
Coronary artery disease

ABSTRACT

Background and aims: Epicardial adipose tissue (EAT) is a visceral AT, surrounding myocardium and coronary arteries. Its volume is higher in Type 2 diabetic (DM2) patients, associated with cardiovascular disease risk. Lipoprotein lipase (LPL) hydrolyses triglycerides (TG) from circulating lipoproteins, supplying fatty acids to AT, contributing to its expansion. We aimed to evaluate LPL expression and activity in EAT from DM2 and no DM2 patients, and its regulators ANGPTL4, GPIHBP1 and PPARγ levels, together with VLDLR expression and EAT LPL association with VLDL characteristics.
Methods: We studied patients undergoing coronary by-pass graft (CABG) divided into CABG-DM2 (n = 21) and CABG-noDM2 (n = 29), and patients without CABG (No CABG, n = 30). During surgery, EAT and subcutaneous AT (SAT) were obtained, in which LPL activity, gene and protein expression, its regulators and VLDLR protein levels were determined. Isolated circulating VLDLs were characterized.
Results: EAT LPL activity was higher in CABG-DM2 compared to CABG-noDM2 and No CABG ($p = 0.002$ and $p < 0.001$) and in CABG-noDM2 compared to No CABG ($p = 0.02$), without differences in its expression. ANGPTL4 levels were higher in EAT from No CABG compared to CABG-DM2 and CABG-noDM2 ($p < 0.001$). GPIHBP1 levels were higher in EAT from CABG-DM2 and CABG-noDM2 compared to No CABG ($p = 0.04$). EAT from CABG-DM2 presented higher PPARγ levels than CABG-noDM2 and No CABG ($p = 0.02$ and $p = 0.03$). No differences were observed in VLDL composition between groups, although EAT LPL activity was inversely associated with VLDL-TG and TG/protein index ($p < 0.05$).
Conclusions: EAT LPL regulation would be mainly post-translational. The higher LPL activity in DM2 could be partly responsible for the increase in EAT volume.

* Corresponding author. Junín 956, CABA, Argentina.
E-mail address: gaberg@ffyb.uba.ar (G. Berg).

https://doi.org/10.1016/j.atherosclerosis.2019.06.915
Received 14 March 2019; Received in revised form 27 May 2019; Accepted 27 June 2019
Available online 06 July 2019

1. Introduction

In the last decades, one of the main challenges of the scientific community has been the prevention of cardiovascular disease (CVD), principal cause of death in the Western world, both in diabetic and non-diabetic patients [1]. Besides, metabolic derangements accompanying type 2 diabetes mellitus (T2DM) are associated with an increase in visceral fat deposits, which are prominent in these patients and are considered an independent risk factor for CVD [2]. Visceral adipose tissue (VAT) is a dynamic endocrine organ, which expansion is associated with increased release of free fatty acids (FFA) and pro-inflammatory agents, and decreased production of anti-inflammatory factors. VAT has been widely studied in T2DM patients, and the correlation between excessive adipose tissue deposition and the development of diabetes has been extensively demonstrated [3].

More recently, attention has been directed to epicardial adipose tissue (EAT), a VAT which surrounds and infiltrates the myocardium and coronary arteries. Due to the close anatomical proximity to the heart and the absence of fascial boundaries, EAT may interact locally with the myocardium and coronary arteries through paracrine and vasocrine secretion of pro-inflammatory and pro-atherogenic adipokines [4,5]. Previous studies from our laboratory showed that EAT from coronary patients present smaller adipocytes and higher degree of inflammatory cells [6]. Nowadays, EAT is considered an active adipose tissue with multiple metabolic and endocrine functions that interacts with surrounding tissues [7]. Thus, the metabolic function of EAT has an important role in patients with metabolic syndrome and T2DM. Moreover, an increase in EAT volume has been associated with T2DM independently of other risk factors [8,9], and it has also been proposed as a predictor of T2DM [10,11]. Furthermore, it has been reported that EAT in T2DM patients presents increased pro-inflammatory cytokines expression [12].

Fat deposition in EAT partially depends on the activity of lipoprotein lipase (LPL). LPL belongs to the extracellular lipases family, together with hepatic lipase (HL) and endothelial lipase (EL) [13], and it has predominantly a triglyceride-hydrolase function on chylomicrons and very low density lipoprotein (VLDL), supplying FFA to the adipocyte. LPL is synthesized by parenchymal cells and transported to the luminal side of the capillary endothelium, where it is anchored by non-covalent interactions to the heparan sulfate side chains of membrane proteoglycans and to the recently discovered glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) [14]. LPL expression is regulated by peroxisome proliferator-activated receptors (PPARs), of which PPARγ is highly expressed in adipose tissue, and its stimulation has been demonstrated to increase LPL gene transcription and protein expression [15]. In reference to LPL activity, it is stimulated by apoproteins CII and AV, and inhibited by apoprotein CIII, the three being components of circulating lipoproteins [15]. More recently, the role of angiopoietin-like protein (ANGPTL) has been implicated in LPL post-translational regulation [15]. ANGPTL3 and ANGPTL8 synthesized nearly exclusively by the liver, and ANGPTL4 synthesized mainly by adipose tissue are considered important negative regulators of LPL activity in fasting, thereby determining fat storage [15]. Otherwise, it is known that insulin also modulates LPL behaviour [16]; in accordance, we have previously demonstrated that LPL activity is decreased in post-heparin plasma from obese and insulin-resistant patients [13]. However, controversial effects of insulin on LPL expression and activity have been reported in different tissues, depending on the tissue and the insulin-resistant degree [16].

Recently, it has been demonstrated that differences in EAT volume between coronary and non-coronary patients may not be caused by a differential mRNA expression of fat mobilizing genes such as LPL, hormone sensitive lipase (HSL), adipose tissue glycerol lipase (ATGL), and ANGPTL4 [17]. However, given that gene expression not always represents the functional capacity of the enzymes, our aim was to study the expression and activity of LPL in EAT from coronary patients, with and without T2DM, and non-coronary patients, as well as ANGPTL4, GPIHBP1 and PPARγ levels. To establish possible associations between LPL activity and its main substrate, we also characterized isolated VLDLs from these patients and evaluated the expression of VLDL receptor (VLDLR) in EAT. Finally, as inflammation is known to be associated with LPL behaviour, we assessed macrophages polarization in EAT.

2. Materials and methods

2.1. Subjects

We included 80 patients of both genders, undergoing coronary artery bypass graft (CABG, n = 50) or valve replacement (No CABG, n = 30). CABG patients group included T2DM patients (CABG-DM2, n = 21) and non T2DM patients (CABG-noDM2, n = 29). The patients attended the Cardiac Surgery Division of Hospital de Clinicas José de San Martín, University of Buenos Aires. Clinical data was obtained upon admission to hospital, before surgery. Diagnosis of CABG was based on previous coronary angiograms. Reductions in luminal coronary artery diameters > 70% were considered significant. Previous diagnosis of T2DM was assessed according to the American Diabetes Association [18]. No CABG patients were randomly selected among patients who did not undergo CABG intervention. These patients had no clinical signs of coronary artery disease and showed normal coronary arteries on angiography. The weight and height of each participant were measured and body mass index (BMI) was calculated to evaluate obesity; blood pressure was recorded in all cases.

The following exclusion criteria were considered for both groups: previous heart surgery, concomitant infective diseases, alcohol intake > 20 g/day, recent history of acute illness, hypothyroidism, renal failure, liver disease, and any other condition that may interfere with the results, such as Chagas disease and Human Immunodeficiency Virus + patients.

Written informed consent was required from all the participants before inclusion in the study. The study was performed in accordance to the ethical guidelines of the Declaration of Helsinki of the World Medical Association for medical studies in humans. The study was approved by the Ethical Review Committee of the Faculty of Pharmacy and Biochemistry, University of Buenos Aires and by the Ethical Review Committee of the Hospital de Clínicas José de San Martín.

2.2. Blood collection

After 10–12 h overnight fast, before cardiovascular surgery, peripheral venous blood samples were drawn. Serum was kept at 4 °C within 48 h for the evaluation of glucose, lipids and lipoproteins profile, apolipoprotein AI and B100, or stored at −70 °C for further determination of FFA, insulin and VLDL isolation.

2.3. Adipose tissue biopsies

EAT (~0.1–0.5 g) and subcutaneous adipose tissue (SAT, ~1.0 g) samples were obtained before starting extracorporeal circulation. EAT biopsies were collected from the area near the proximal tract of the right coronary artery and superficial SAT samples were harvested from the anterior thorax. Tissue samples were aliquoted and immediately frozen in liquid nitrogen until analysis.

2.4. Biochemical determinations

Total cholesterol, triglycerides (TG), and fasting glucose were measured using commercial enzymatic kits (Roche Diagnostics, Germany) in a Cobas C-501 autoanalyzer; intra-assay coefficient of variation (CV) < 1.9%, inter-assay CV < 2.4%. High density and low-density lipoprotein cholesterol (HDL-C and LDL-C) were determined by homogeneous colorimetric method; intra-assay CV < 2.0% and inter-

assay CV < 2.5%, respectively, and Non HDL-cholesterol (Non HDL-C) was calculated as total cholesterol minus HDL-C. Serum apolipoproteins A-I (apoA-I) and B-100 (apoB-100) were determined by immunoturbidimetry (Roche Diagnostics, Germany); intra-assay CV < 1.9%, and inter-assay CV < 2.5% for both parameters. FFA were determined by a spectrophotometric method (Randox, UK); intra-assay CV < 2.6% and inter-assay CV < 3.9%. Insulin was measured with Immulite/Immulite 1000 Insulin (Siemens, USA); intra-assay CV < 2.6%, and inter-assay CV < 3.9%. To estimate insulin-resistance (IR), the homeostasis model assessment for insulin resistance (HOMA-IR) index [19] and the TG/HDL-C index were calculated. Remnant lipoproteins cholesterol (RLP-C) was calculated as Total cholesterol –LDL-C - HDL-C.

2.5. VLDL isolation

VLDL (density < 1.006 g/ml) was isolated by sequential preparative ultracentrifugation [20] in a Beckman XL-90 using a fixed-angle rotor type 90 Ti at 105.000 × *g*, for 18 h, at 18 °C. Purity of lipoprotein was tested by agarose gel electrophoresis. Isolated VLDL composition was characterized by the following parameters: cholesterol and TG, using the previously described methods, phospholipids by the Fiske-Subbarow method [21] and proteins by the Lowry method [22].

2.6. LPL activity

LPL activity assay in EAT and SAT was performed in a blinded manner and each sample was evaluated in three different replicates.

LPL activity was determined by measuring the oleic acid produced by the enzyme catalyzed hydrolysis of an emulsion containing [^{3}H]-triolein (American Radiolabeled Chemicals, USA) according to Nilsson-Ehle method [23]. A piece of adipose tissue was cut into 1–2 mg fractions, and incubated in Krebs-Ringer Buffer containing 2.5 IU of Heparin (Sigma Aldrich, USA) and 1 g% of BSA (Sigma Aldrich, USA), for 40 min at 37 °C. The assay mixture for LPL activity contained labelled and unlabeled Triolein (Sigma Aldrich, USA) (1.3 mmol/ml of glyceryltrioleate with a specific activity of 10 × 10^{6}cpm/mmol), mixed with 0.11 mmol/ml of L-lysophosphatidylcholine (Sigma Aldrich, USA), 4% bovine serum albumin (Sigma Aldrich, USA), 10% v/v of human serum as source of apoC-II, in 0.2 M buffer Tris–HCl pH 8.0 with NaCl 0.3 M. This mixture was incubated with an aliquot of the extract for 1 h at 37 °C. After incubation, the reaction was stopped and the released fatty acids were isolated by extraction with a mixture of Methanol/Chloroform/Heptane (1.45:1.25:1 by vol) and carbonate-borate buffer, pH 10.5. The labelled fatty acids were quantified by counting with a Liquid Scintillation Analyzer (Packard TRI-CARB 2100; Packard Instruments, Meridian, CT). LPL activity was calculated as the mols of fatty acids released per minute per milligram of tissue, and expressed as μ International Units (IU, as 1 IU = 1 μmol of fatty acid released per minute) per mg.

2.7. LPL, ANGPTL4, GPIHBP1, PPARγ and VLDLR protein levels

LPL and its regulators levels were assessed by Western Blot.

EAT and SAT were homogenized in 20 mM Tris buffer, pH 7.4, containing 150 mM NaCl, 1% Triton X-100 and 2% protease inhibitor cocktail (Sigma Aldrich, USA). Tissue homogenates were centrifuged and protein concentrations were determined by Lowry's method in the supernatant. For LPL, ANGPTL4, PPARγ, VLDLR and β-actin, 30 μg of protein were separated in a 12% sodium dodecyl sulfate-polyacrylamide (SDS) gel electrophoresis and electroblotted onto polyvinylidene difluoride membrane. For GPIHBP1, 15% SDS gel was used. Blots were blocked with 5% skim milk for 1 h and incubated overnight at 4 °C with a polyclonal rabbit IgG antibody anti-LPL (4 μg/ml, Thermo Fisher Scientific, USA), anti-ANGPTL4 (1:150, Sigma Aldrich, USA), anti-PPARγ (1:150, Santa Cruz Biotechnology, Germany), anti-GPIHBP1 (1:500, Abcam, UK), anti-VLDLR (1:200, Abcam, UK), or anti-β-actin (1:300, Sigma Aldrich, USA). After washing with Tris Buffer Saline-Tween 0.1%, the blots were incubated with HRP conjugated secondary antibody (1:4000, BioRad, USA) for 1.30 hs at room temperature. The specific signals were visualized using the ECL Western Blotting Analysis System (ThermoScientific, USA) enhanced chemiluminescence system. LPL (63 kDa), ANGPTL4 (45 kDa), GPIHBP1 (21 kDa), PPARγ (64 kDa), VLDLR (96 kDa) and β-actin (42 kDa) bands were identified by the use of pre-stained molecular weight standards (BioRad, USA). The relative intensity of protein signal was quantified by densitometric analysis using Fluorchem program (Alpha Innotech Corp, USA). Results are expressed as LPL, ANGPTL4, GPIHBP1, PPARγ or VLDLR protein/actin protein ratio.

2.8. LPL and M1/M2 markers mRNA levels

In a subgroup of patients in which the sample size was enough to perform the RNA extraction, the expression of LPL was analyzed by RT-qPCR, as well as MCP-1, IL-6 and IL-12, as M1 profile markers and TGF-β, as M2 profile marker.

2.8.1. mRNA purification

Total RNA was obtained from EAT and SAT homogenates using Trizol reagent (ThermoScientific, USA), treated with DNAse (Life Technologies, USA). Total RNA was reverse transcribed using Expand Reverse Transcriptase (Promega Corporation), according to the instructions.

2.8.2. Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

mRNA expression was determined using 5X HOT FIREPOL EVAGREEN qPCR (Solis BioDyne, Estonia) in a StepOnePlus Real-Time PCR System. Parameters were: 52 °C for 2 min, 95 °C for 15 min, and 40 cycles at 95 °C for 15 s, specific Tm °C for 30 s and 72 °C for 1 min. Normalization was carried out using Actin cDNA. Quantification was performed using the comparative threshold cycle (Ct) method, as all the primer pairs (target gene/reference gene) were amplified using comparable efficiencies (relative quantity, 2-ΔΔCt) [24].

2.8.3. Primer sequences

	Forward primer 5′-3′	Reverse primer 5′-3′	MT (°C)
β-Actin	GTGGGGCGCCCCAGGCACCA	CGGTTGGCCTTGGGGTTCAGGGGG	65
LPL	TCAACTGGATGGAGGAGGAGT	CAGGAGAAAGACGACTCGGG	63
MCP-1	TGGTCCCCTGTGCCTTGA	CTTGAAGATCACAGCTTCTTTGG	63
IL-6	TATTAGAGTCTCAACCCCCAATAAA	ACCAGGCAAGTCTCCTCATT	60
IL-12	CTCCTGGACCACCTCAGTTT	TGGTGAAGGCATGGGAACAT	63
TGF-β	ATGGAGAGAGGACTGGGGAT	TGGTCCCCTGTGCCTTGA	63

MT: melting temperature.

2.9. Statistical analysis

Data are presented as mean ± SD or median (range) according to normal or skewed distribution, respectively. Data distribution was tested by the Kolmogorov and Smirnov test. Differences between groups were tested using χ^2, ANOVA + Bonferroni or Kruskal-Wallis + Mann-Whitney tests, according to the data distribution. Statistical comparisons between EAT and SAT from the same group were tested by paired Student *t*-test. To verify the differences observed between groups, we performed an analysis of covariance (ANCOVA), controlling for necessary confounders. Only complete sets (i.e. pairs) of EAT and SAT results are reported. Pearson or Spearman analysis, for parametric or non-parametric variables, were used to determine correlations between parameters. Prior to the onset of the study, the number of patients required for detecting differences of at least 1 Standard Deviation, with

and statistical power of 80% and an $\alpha = 0.05$, were determined. According to this, for all the studied parameters the number of cases should not be less than 15. The SPSS 19.0 (Chicago, USA) and GraphPad Prism 5.01 (La Jolla, USA) software packages were used for statistical analysis. A two-tailed $p < 0.05$ was considered significant.

3. Results

3.1. Clinical and biochemical characteristics of the study population

Clinical and biochemical characteristics of the study population are shown in Table 1. Gender distribution was different between CABG-DM2 and No CABG, being the percentage of men higher in the diabetic group. Patients with CABG-DM2 presented higher systolic blood pressure than CABG-noDM2, without differences in the other clinical characteristics between groups. Regarding medication, most of the patients were on treatment with statins, aspirin, β-blockers or ACE inhibitors. The consumption of statins and aspirin in patients with CABG was higher than in the No CABG group, without differences between CABG-DM2 and CABG-noDM2. Besides, only the CABG-DM2 group was under treatment with metformin (Table 1) and in this group four patients were under insulin treatment, two under sulfonylureas treatment and two under DPP-4 inhibitors treatment.

When evaluating the lipid-lipoprotein profile, an increase in TG levels was observed in the CABG group with and without DM2 compared to No CABG. Finally, as expected, the CABG-DM2 group presented higher levels of glucose and insulin as well as higher TG/HDL-C and HOMA indexes compared to the other groups (Table 1).

3.2. Lipoprotein characteristics

In reference to VLDL composition, there were no differences in any of its components between the CABG and No CABG group. When TG/ApoB, TG/protein and lipid/protein indexes were calculated, a tendency to higher values was observed in the CABG group in comparison to No CABG. No differences were observed in these parameters between DM2 and no DM2 patients (Table 2).

Table 1
Clinical and biochemical characteristics of the population under study.

	No CABG	No DM2 (n = 29)	CABG
	(n = 30)		DM2 (n = 21)
Age (years)	71 ± 7	67 ± 9	64 ± 9
W/M	12/18	7/22	1/20[1]
SBP (mmHg)	130 (90–180)	129 (100–171)	140 (106–168)[2]
DBP (mmHg)	73 (50–97)	70 (40–90)	80 (48–96)
BMI (kg/m^2)	28.0 ± 5.4	27.1 ± 2.9	27.3 ± 3.7
HT (%)	67	66	57
Ex smokers (%)	50	59	62
Metformin (%)	0	0	75[3]
Insulin (%)	0	0	19[4]
Statins (%)	41	74[5]	75[6]
ACEI (%)	54	56	45
β-Blockers (%)	60	62	52
Aspirin (%)	36	78[7]	70[8]
Triglycerides (mmol/L)	1.10 (0.45–2.12)	1.60 (0.64–3.21)[9]	2.20 (0.69–3.15)[10]
Total cholesterol (C) (mmol/L)	3.19 (1.66–5.62)	3.29 (2.38–6.79)	3.32 (1.71–5.02)
LDL-C (mmol/L)	1.79 (0.73–2.98)	1.86 (1.24–3.34)	2.02 (0.54–3.11)
HDL-C (mmol/L)	0.91 ± 0.26	0.83 ± 0.21	0.80 ± 0.21
Non HDL-C (mmol/L)	2.07 (1.30–4.30)	2.38 (1.48–7.17)	2.54 (0.85–4.45)
RLP-C (mmol/L)	0.39 (0.03–1.49)	0.49 (0.08–1.48)	0.47 (0.05–1.55)
ApoA-I (g/L)	0.93 ± 0.29	0.87 ± 0.20	0.90 ± 0.14
ApoB-100 (g/L)	0.60 ± 0.15	0.77 ± 0.19	0.66 ± 0.27
FFA (mmol/L)	1.20 ± 0.69	0.86 ± 0.30	1.03 ± 0.60
Glycaemia (mmol/L)	5.27 (4.27–7.77)	5.38 (4.16–7.38)	7.44 (4.00–14.71)[11]
Insulinemia (pmol/L)	32.64 ± 18.75	26.39 ± 11.11	47.92 ± 35.42[12]
TG/HDL-C	2.9 (1.1–5.0)	3.1 (1.2–6.1)	3.9 (1.7–11.0)[13]
HOMA-IR	1.22 ± 0.75	1.41 ± 0.90	2.44 ± 1.39[14]

Results are expressed as mean ± standard deviation or median (range).
[1] $p = 0.020$ *vs.* No CABG.
[2] $p = 0.030$vs CABG No DM.
[3] $p < 0.001$ *vs.* CABG No DM2 and No CABG.
[4] $p = 0.040$ *vs.* CABG No DM2 and No CABG.
[5] $p = 0.005$ *vs.* No CABG.
[6] $p = 0.005$ *vs.* No CABG.
[7] $p = 0.010$ *vs.* No CABG.
[8] $p = 0.010$ *vs.* No CABG.
[9] $p = 0.005$ *vs.* No CABG.
[10] $p < 0.001$vs No CABG.
[11] $p = 0.048$ *vs.* CABG No DM2 and No CABG.
[12] $p = 0.007$ *vs.* CABG No DM2.
[13] $p = 0.010$ *vs.* CABG No DM2 and No CABG.
[14] $p = 0.045$ *vs.* CABG No DM2 and No CABG. Differences in means or medians between groups were evaluated using ANOVA + Bonferroni or Kruskal-Wallis + Mann-Whitney tests according to data distribution. Percentages differences were evaluated with χ^2 test.
W/M: women/men, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: body mass index, HT: hypertension, ACEI: angiotensin converting enzyme inhibitors, RLP: remnant lipoproteins, FFA: free fatty acids.

Table 2
Isolated VLDL characteristics.

	No CABG	CABG	
		No DM2	DM2
Triglycerides (mmol/L)	0.25 ± 0.14	0.28 ± 0.15	0.21 ± 0.14
Cholesterol (mmol/L)	0.18 (0.03–0.28)	0.18 (0.03–0.78)	0.13 (0.03–0.91)
Phospholipids (mmol/L)	0.06 (0.03–0.10)	0.06 (0.03–0.13)	0.06 (0.03–0.10)
Proteins (g/L)	0.10 ± 0.06	0.14 ± 0.07	0.13 ± 0.08
ApoB (g/L)	0.016 ± 0.008	0.015 ± 0.009	0.020 ± 0.009
TG/ApoB	14.6 ± 4.5	19.1 ± 10.0[1]	17.1 ± 5.2[1]
TG/proteins	1.9 ± 0.9	2.3 ± 1.1	2.3 ± 1.1[1]
Cholesterol/TG	0.3(0.1–0.8)	0.3(0.1–0.5)[1]	0.3(0.4–0.8)
Lipids/proteins	2.7 ± 1.0	3.2 ± 1.5[1]	3.1 ± 1.4[1]

Results are expressed as mean ± standard deviation or median (range).
[1]$p = 0.080$ *vs*. No CABG. Differences in means or medians between groups were evaluated using ANOVA + Bonferroni or Kruskal-Wallis + Mann-Whitney tests according to data distribution.

3.3. LPL levels

No differences were observed in LPL gene and protein expression among groups nor tissues (Fig. 1A and B).

LPL protein levels were not associated with lipid profile parameters nor VLDL characteristics in EAT nor SAT.

3.4. Lipoprotein lipase activity

In EAT, LPL activity was increased in the CABG compared to No CABG group ($p = 0.020$). Moreover, CABG-DM2 patients presented higher LPL activity in EAT compared to CABG-noDM2 and No CABG (Fig. 1C). The differences between CABG-DM2 and No CABG remained significant after adjusting for gender (F = 5.42, $p = 0.030$), aspirin consumption (F = 11.18, $p = 0.001$) and statins treatment (F = 10.69, $p = 0.001$). Moreover, CABG-noDM2 showed higher enzymatic activity than No CABG; given that no differences in gender were observed between these two groups, results were adjusted only by aspirin (F = 4.19, $p = 0.049$) and statins (F = 3.98, $p = 0.047$) treatments, being the differences still significant. Besides, the activity of LPL was significantly higher in EAT than SAT in all groups (Fig. 1C). Regarding LPL activity in SAT, no significant differences were observed between groups (Fig. 1C).

LPL activity from EAT was inversely associated with circulating TG levels (No CABG: r = −0.302, $p = 0.040$; CABG-noDM2: r = −0.346, $p = 0.040$; CABG-DM2: r = −0.481, $p = 0.045$) (Fig. 2A) and with RLP-C (No CABG: r = −0.245, $p = 0.048$; CABG-noDM2: r = −0.441, $p = 0.011$; CABG-DM2: r = −0.366, $p = 0.044$) (Fig. 2B). No associations between SAT LPL activity and lipid profile parameters were found.

Regarding EAT LPL contribution to lipoprotein characteristics, inverse associations with VLDL TG content as well as with TG/ApoB and TG/protein VLDL indexes were found (Table 3).

3.5. ANGPTL4 levels

ANGPTL4 levels were higher in EAT from No CABG compared to CABG-DM2 and CABG-noDM2, and in SAT compared to EAT in all groups (Fig. 1D). Differences between CABG-DM2 and No CABG remained significant after adjusting for gender (F = 10.16, $p = 0.001$), aspirin consumption (F = 9.15, $p = 0.002$) and statins treatment (F = 12.35, $p = 0.001$). Besides, differences between CABG-noDM2 and No CABG remained significant after adjusting for aspirin (F = 8.52, $p = 0.002$) and statins consumption (F = 8.42, $p = 0.001$).

In EAT, ANGPTL4 was inversely associated with LPL activity in the whole studied population (r = -0.705, $p < 0.001$), and this association remained significant in each group (No CABG: r = -0.669, $p = 0.012$; CABG-No DM2: r = -0.702, $p = 0.002$; CABG-DM2: r = -0.475, $p = 0.049$) (Fig. 2C).

In SAT, ANGPTL4 expression was lower in CABG-DM2 compared to CABG-noDM2 and No CABG ($p = 0.020$) (Fig. 1D). The latter difference remained significant after correction for gender (F = 4.01, $p = 0.048$), aspirin consumption (F = 4.21, $p = 0.045$) and statins treatment (F = 3.67, $p = 0.049$).

3.6. GPIHBP1 levels

GPIHBP1 levels were higher in EAT from CABG-DM2 and CABG-noDM2 compared to No CABG, and these differences remained significant after adjusting for the corresponding co-variables: gender (CABG-DM2 *vs.* No CABG: F = 6.30, $p = 0.046$), aspirin (CABG-DM2 *vs*. No CABG: F = 3.76, $p = 0.044$; CABG-noDM2 *vs*. No CABG: F = 4.84, $p = 0.018$) and statins (CABG-DM2 *vs*. No CABG: F = 3.76, $p = 0.045$; CABG-noDM2 *vs*. No CABG: F = 4.97, $p = 0.017$) treatment. There were not differences in GPIHBP1 levels between CABG-noDM2 and CABG-DM2, neither between EAT and SAT in any of the studied groups (Fig. 1E).

EAT GPIHBP1 levels were directly associated with EAT LPL activity (r = 0.306, $p = 0.046$), and this association remained significant in each group (No CABG: r = 0.568, $p = 0.043$; CABG-No DM2: r = 0.580, $p = 0.014$; CABG-DM2: r = 0.611, $p = 0.046$) (Fig. 2D).

3.7. PPARγ levels

EAT from CABG-DM2 presented higher PPARγ levels than EAT from CABG-noDM2 and No CABG. The latter difference remained significant after correction for gender (F = 4.66, $p = 0.019$), aspirin consumption (F = 4.10, $p = 0.031$) and statins treatment (F = 4.15, $p = 0.043$). No differences in PPARγ from SAT were observed between groups (Fig. 1F).

In EAT, PPARγ levels were directly associated with LPL activity (r = 0.539, $p < 0.001$) in the whole population, and this association remained significant in all groups (No CABG: r = 0.757, $p < 0.001$; CABG-No DM2: r = 0.450, $p = 0.048$; CABG-DM2: r = 0.837, $p = 0.005$) (Fig. 2E).

3.8. VLDLR levels

No differences were observed in VLDLR levels in EAT nor SAT between groups. VLDLR levels were lower in SAT than EAT in the CABG group. When dividing the CABG group, the difference was only significant in CABG-noDM2 (Supplementary Fig. 1).

EAT VLDLR levels were inversely associated with TG-VLDL (r = -0.382, $p = 0.044$) in the whole studied population, and this associations remained significant in CABG-noDM2 (r = -0.689, $p = 0.020$) and CABG-DM2 (r = -0.644, $p = 0.047$).

3.9. Macrophage polarization in EAT

Higher levels of MCP-1 and IL-6 were verified in EAT from CABG patients compared to No CABG (MCP-1: 9.72 (0.17–13.01) *vs* 1.27 (0.01–3.20) RU, $p = 0.040$, respectively. IL-6: 9.59 ± 4.02 *vs* 0.83 ± 0.23 RU, $p = 0.035$, respectively). When dividing CABG patients according to DM2, although higher values of these markers were found in DM2 patients (MCP-1: 2.16 (0.17–5.22) in CABG-noDM2 *vs* 3.11 (0.42–13.01) in CABG-DM2 (relative units to *actin* mRNA, $p = 0.800$. IL-6: 3.25 ± 1.00 *vs* 2.83 ± 0.78 relative units to *actin* mRNA, $p = 0.786$), the differences did not reach significance. Moreover, a positive association between MCP-1 levels and GPIHBP1 expression in EAT was verified (r = 0.777, $p = 0.005$).

No differences in IL-12 and TGF-β were found among groups.

3.10. LPL behaviour according to insulin-resistance

In EAT, LPL activity was inversely associated with TG/HDL-C index in CABG (r = -0.312, $p = 0.047$) and No CABG group (r = -0.416, $p = 0.041$).

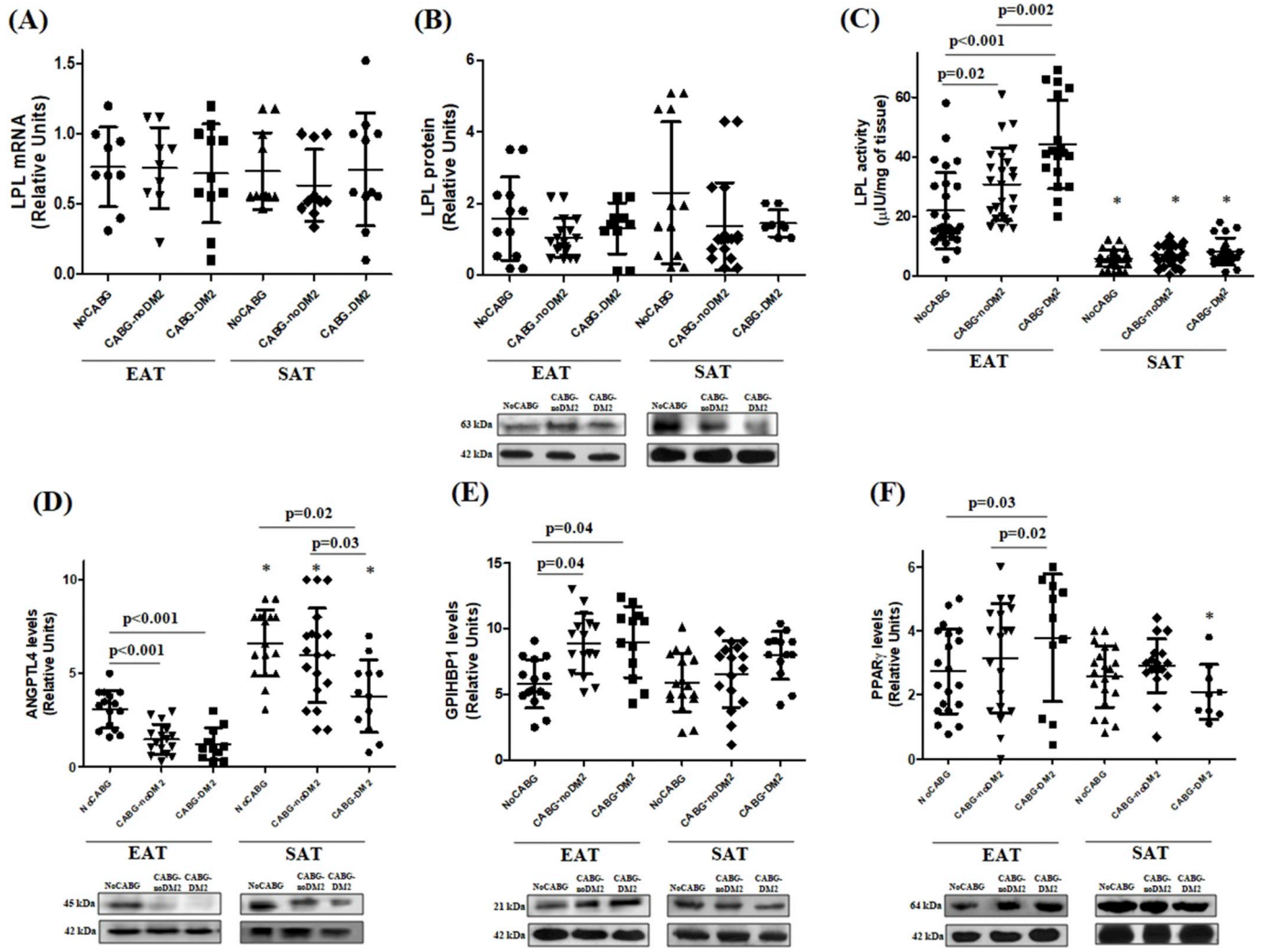

Fig. 1. (A) No differences were observed in *LPL* mRNA expression between groups nor tissues (EAT No CABG: 0.765 ± 0.284; SAT No CABG: 0.736 ± 0.274; EAT CABG-noDM2: 0.758 ± 0.289; SAT CABG-noDM2: 0.635 ± 0.256; EAT CABG-DM2: 0.719 ± 0.352; SAT CABG-DM2: 0.745 ± 0.403, relative units to *actin* mRNA). (B) No differences were observed in LPL protein expression between groups nor tissues (EAT No CABG: 1.57 ± 1.16; SAT No CABG: 2.30 ± 1.98; EAT CABG-noDM2: 1.04 ± 0.54; SAT CABG-noDM2: 1.36 ± 1.22; EAT CABG-DM2: 1.31 ± 0.71; SAT CABG-DM2: 1.44 ± 0.37, relative units to actin). (C) EAT LPL activity was higher in CABG-DM2 compared to CABG-noDM2 and No CABG, and in CABG-noDM2 compared to No CABG (EAT No CABG: 21.89 ± 17.78; EAT CABG-noDM2: 30.71 ± 12.24; EAT CABG-DM2: 44.06 ± 14.81, μIU/mg of tissue). In SAT, no differences in LPL activity were observed between groups (SAT No CABG: 5.70 ± 2.94; SAT CABG-noDM2: 6.84 ± 3.46; SAT CABG-DM2: 8.10 ± 4.62, μIU/mg of tissue). In each group, EAT LPL activity was higher than SAT LPL activity, $^{*}p < 0.001$. (D) In EAT, ANGPTL4 levels were lower in CABG-noDM2 and CABG-DM2 than in No CABG (EAT No CABG: 3.10 ± 1.01; EAT CABG-noDM2: 1.48 ± 0.81; EAT CABG-DM2: 1.24 ± 0.86, relative units to actin). In SAT, ANGPTL4 levels were lower in CABG-DM2 compared to CABG-noDM2 and No CABG (SAT No CABG: 6.63 ± 1.77; SAT CABG-noDM2: 5.97 ± 2.51; SAT CABG-DM2: 3.79 ± 1.95, relative units to actin). In each group, EAT ANGPTL4 expression was lower than SAT ANGPTL4 expression, $^{*}p < 0.001$. (E) GPIHBP1 levels in EAT were higher in CABG-noDM2 and CABG-DM2 than in No CABG (EAT No CABG: 5.82 ± 1.87; EAT CABG-noDM2: 8.87 ± 2.29; EAT CABG-DM2: 9.00 ± 2.71, relative units to actin). No differences were found in GPIHBP1 levels in SAT between groups (SAT No CABG: 5.91 ± 2.21; SAT CABG-noDM2: 6.54 ± 2.51; SAT CABG-DM2: 7.97 ± 1.82, relative units to actin). No differences were observed in GPIHBP1 levels between EAT and SAT in any of the studied groups. (F) In EAT, PPARγ levels were higher in CABG-DM2 compared to CABG-noDM2 and No CABG (EAT No CABG: 2.74 ± 1.32; EAT CABG-noDM2: 3.15 ± 1.71; EAT CABG-DM2: 3.79 ± 1.99, relative units to actin). No differences were observed in PPARγ levels in SAT between groups (SAT No CABG: 2.57 ± 0.96; SAT CABG-noDM2: 2.91 ± 0.84; SAT CABG-DM2: 2.09 ± 0.86, relative units to actin). In CABG-DM2, EAT PPARγ expression was higher than in SAT, $^{*}p < 0.001$.
Results are expressed as mean ± standard deviation. Mean differences between groups were analyzed using ANVOA + Bonferoni tests; mean differences between tissues were analyzed using paired Student *t*-test. ANGPTL4: angiopoietin-like 4; CABG: coronary by-pass graft; DM2: diabetes mellitus 2; EAT: epicardial adipose tissue; GPIHBP1: glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1; IU: International Units; LPL: lipoprotein lipase; PPARγ: peroxisome-proliferator activated receptor γ; SAT: subcutaneous adipose tissue.

When dividing the CABG group according to TG/HDL-C tertiles, no significant differences were observed in EAT LPL activity among groups, nor in LPL regulators levels; however, LPL activity showed a tendency ($p = 0.081$) to decrease in the highest TG/HDL-C tertile, accompanied by a decrease in LPL expression, GPIHBP1, VLDLR and PPARγ levels and an increase in ANGPTL4 expression (Supplementary Fig. 2).

No associations were observed between these parameters and HOMA index.

When patients from the CABG-DM2 group were divided according to being or not under metformin treatment, treated patients presented higher expression of PPARγ in EAT ($p = 0.048$) and, concomitantly, a tendency ($p = 0.062$) to increased LPL activity and GPIHBP1 expression. ANGPTL4 levels were not modified by treatment with metformin. Regarding LPL expression, a tendency ($p = 0.080$) to lower levels was observed in CABG-DM2 without metformin treatment (Supplementary Fig. 3).

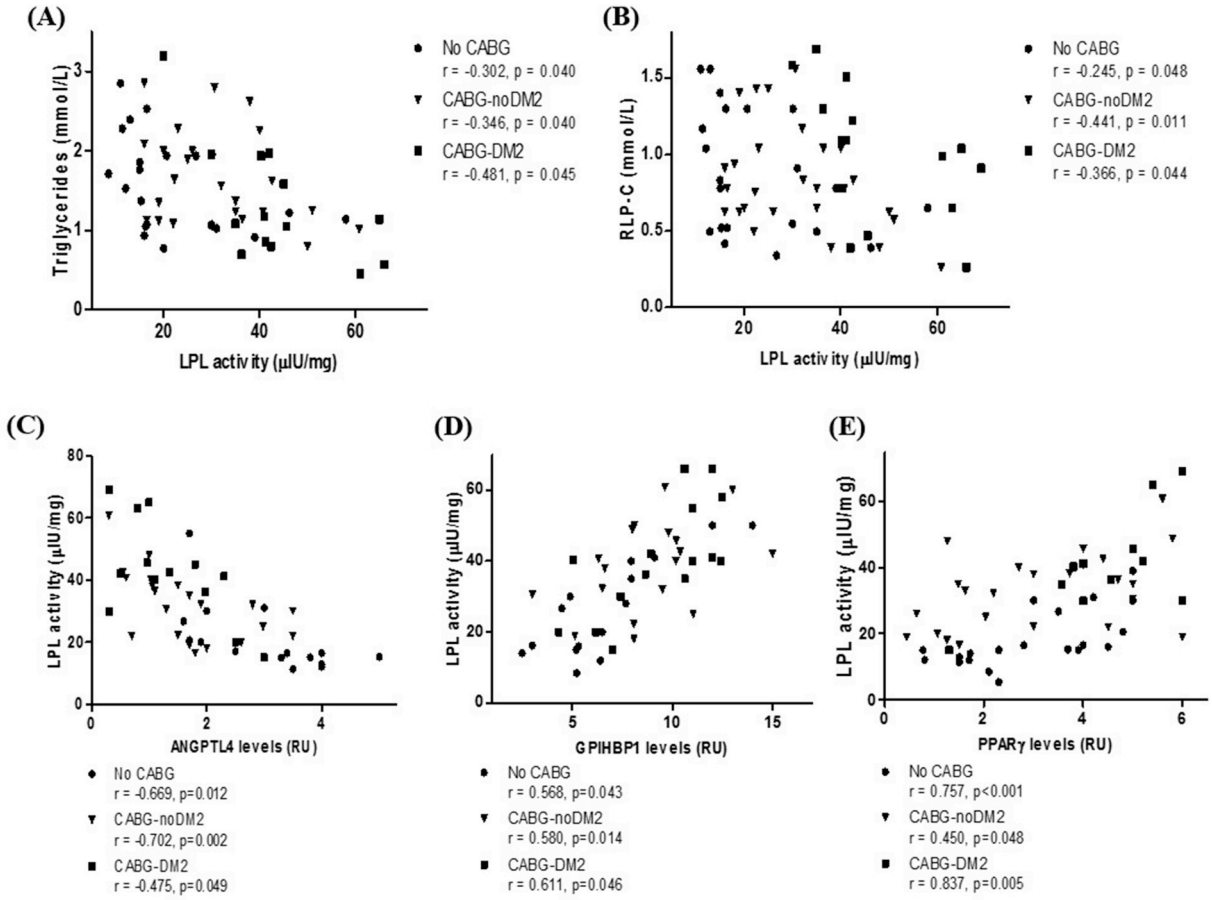

Fig. 2. Correlation plots between EAT LPL activity, lipid profile (A and B) and LPL regulators expression (C, D and E).
The three groups are differentially identified. Pearson or Spearman test were used according to data distribution. ANGPTL4: angiopoietin-like 4; CABG: coronary bypass graft; DM2: diabetes mellitus 2; EAT: epicardial adipose tissue; GPIHBP1: glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1; IU: International Units; LPL: lipoprotein lipase; PPARγ: peroxisome-proliferator activated receptor γ; RLP-C: remnant lipoproteins cholesterol; SAT: subcutaneous adipose tissue; TG: triglycerides.

Table 3
Correlations between EAT and SAT LPL activity and VLDL characteristics.

VLDL	LPL activity	
	EAT R (p)	*SAT R (p)*
Triglycerides	−0.472(0.040)	−0.130 (0.751)
Cholesterol	−0.205 (0.297)	0.156 (0.147)
Phospholipids	−0.325 (0.230)	0.196 (0.418)
Proteins	0.022 (0.534)	0.254 (0.584)
TG/ApoB	−0.440(0.048)	−0.122 (0.576)
TG/proteins	−0.415 (0.048)	−0.395 (0.460)
Cholesterol/TG	0.102 (0.851)	0.056 (0.979)
Lipids/proteins	−0.229 (0.553)	−0.115 (0.279)

Pearson or Spearman test were performed, according to data distribution.

To verify whether insulin, sulfonyulreas and/or DPP-4 inhibitors treatments were interfering with the results, statistical analysis was performed excluding patients from the CABG-DM2 group. All statistically significant differences verified in the studied parameters were conserved.

4. Discussion

In the present study, we report, for the first time, LPL activity in EAT from coronary patients, with and without diabetes. An increase in EAT LPL activity was verified in coronary patients compared to non coronary, and in coronary diabetic patients, compared to coronary non diabetic. This activity was accompanied by a concomitant behaviour of its main regulators, an increase in GPIHPB1 levels and a decrease in ANGPTL4 levels.

It has been previously demonstrated that an increase in EAT volume is directly associated with T2DM [8,9]. Moreover, metabolic derangements in T2DM patients are significantly linked to EAT, thus it has been proposed as an independent risk factor for CVD in diabetes [25].

One of the principal mechanisms responsible for the increase in EAT volume could be the higher influx of fatty acids to the tissue. LPL, through the hydrolysis of TG from circulating lipoproteins, could be involved in the supply of fatty acids.

Previous findings have reported that the increase in EAT volume would not be mediated by differences in fat mobilizing enzymes gene expression, such as LPL [17], however, gene expression not necessarily represents the functional capacity of enzymes. Thus, in this study, we have deepen the knowledge of LPL behaviour in EAT, including gene and protein expression and activity. Regarding LPL gene and protein expression, the lack of differences among groups observed in this study suggests that the behaviour of EAT LPL in diabetes would be mainly determined at its post-translational levels. In this regard, LPL activity, ANGPTL4 and GPIHBP1 expression in EAT were evaluated.

Even though LPL has been extensively studied in diabetes, it is still a controversy, given the complex regulation of the enzyme and its differential expression among tissues. Chiu et al. reported an increase in cardiac LPL activity in streptozotocin diabetic mice [26]. However, in a previous study, we observed diminished LPL activity in heart and visceral adipose tissue from insulin resistant rats [27]. In line with this,

studies in patients with metabolic syndrome showed a decrease in post heparin plasma LPL activity, inversely associated with the insulin resistant degree [13]. Nevertheless, the activity of this enzyme has not been previously reported in EAT. We found that, despite its expected inverse association with insulin resistance, patients with diabetes presented higher LPL activity in EAT than non diabetic ones. Thus, other factors beyond insulin resistance could be regulating LPL behaviour in EAT, and the increase in LPL activity could be partly responsible for the increase in EAT volume reported in diabetic patients.

Recently, GPIHBP1 was identified as the necessary protein to anchor LPL to the cell surface, allowing TG hydrolysis [28]. GPIHBP1 is highly expressed in the endothelial cells of adipose tissue and heart, showing high similarity to the tissue expression profiles of LPL [29]. However, its expression has not been yet evaluated in EAT. Ruge et al. did not find insulin-dependent changes in abdominal SAT *GPIHBP1* gene expression [16]. Moreover, Surendran et al. showed inverse associations of *GPIHBP1* mRNA with HOMA, glucose, insulin and BMI [30]. In our study, despite EAT GPIHBP1 levels were inversely associated with TG/HDL-C index, they were increased and directly associated with LPL activity in EAT from diabetic patients.

Another post-translational LPL regulator is ANGPTL4, which is proposed to inhibit LPL activity by dissociating its subunits [15], promoting its intracellular degradation [31] and/or the unfolding of active LPL monomers [32]. Previous studies reported increased concentrations of circulating ANGPTL4 in subjects with obesity and T2DM [33], and variations in *ANGPTL4* gene inversely correlate with plasma TG levels [34–36]. ANGPTL4 expression in different AT has been previously reported [37] as well as its gene expression in EAT, without differences between coronary and non coronary patients [17]. However, its protein levels have not been evaluated in EAT, neither in coronary artery disease nor in diabetes. We demonstrated higher ANGPTL4 protein levels in SAT compared to EAT, as previously reported by Dijk et al. [37]. Even more, the authors showed a negative association between ANGPTL4 and LPL protein levels in SAT [37], and in our study, a similar behaviour was verified between ANGPTL4 protein and LPL activity. Taken together, these results support ANGPTL4 role as an enzyme inhibitor, still the underlying mechanisms need to be clarified.

In reference to PPARγ expression, it is known that this transcription factor regulates several proteins involved in lipid metabolism, among which are LPL, GPIHBP1 and ANGPTL4 [38,39]. In our study, the direct association with LPL activity, despite the lack of association with the enzyme expression, would suggest that additional PPARγ-regulated pathways could be increasing LPL activity. Further studies are necessary to elucidate PPARγ role in EAT.

Given that inflammation has been linked with EAT expansion [40], a possible association with LPL behaviour could be considered. Controversial results have been published about inflammation and LPL [41,42]. In our study, the higher levels of M1 markers observed in CABG patients suggest a pro-inflammatory macrophages profile, and the association between MCP-1 and GPIHBP1 levels would subscribe to the theory of a positive regulation of LPL in an inflamed tissue.

It is well known that in insulin resistant states, VLDL levels are frequently increased, as a consequence of a higher synthesis, together with a decreased catabolism of the lipoprotein [13,43]. In our study, the higher TG/apoB index would indicate the presence of TG-enriched VLDL in coronary patients, despite the higher LPL activity in EAT. It should not be ruled out that VLDL characteristics would be determined by its hepatic synthesis and LPL activity contribution from different tissues. Beyond this, the expected inverse associations between LPL activity and VLDL TG content were observed. Regarding lipoprotein receptors in EAT, we verified the VLDLR presence in the tissue. It has been previously reported that VLDLR mRNA levels were higher in EAT from diabetic patients, without differences in its protein expression [44]. In line with this, our results did not show differences in EAT VLDLR protein expression among groups, however, we observed an inverse association with VLDL TG content. These results indicate that EAT LPL and VLDLR would partly contribute to circulating VLDL characteristics.

Concerning metformin contribution to EAT metabolism, an increase in LPL activity in T2DM patients that were under metformin treatment was verified, together with an increase in PPARγ and GPIHBP1 expression. It has been reported that metformin regulates PPARγ expression [45], and increases plasma LPL activity in diabetic patients [46]. Our results are in line with the reported insulin-sensitizing role of metformin in adipose tissue, favouring mechanisms that are involved in fatty acids supply to the tissue, even though it has been reported that metformin treatment does not cause modifications in EAT volume [47]. Further studies are necessary to elucidate the final effect of metformin treatment in EAT behaviour.

We acknowledge some limitations in this study. First, the sample size in each group was rather small; nevertheless, the tissue nature and the difficulty for its collection must be highlighted, principally in non coronary patients. Second, the assessment of EAT volume in patients included in this study would have been interesting and would have added valuable information. Regarding LPL regulation by PPARγ, evaluating its intracellular localization, beyond its expression, would allow a better understanding of its contribution to EAT metabolism. Finally, given the observational design of this study, it is not possible to state the direct role of LPL activity in EAT behaviour. Further studies, aiming to elucidate the direct effect of lipids metabolism in EAT, would be necessary.

Our study highlights the importance of having evaluated EAT LPL activity, beyond its gene and protein expression, revealing the final behaviour of this enzyme in EAT and its possible contribution to the tissue expansion. Moreover, given the complex regulation of LPL, we report for the first time, the role of ANGPTL4, GPIHBP1 and PPARγ in EAT metabolism, and their implication in CVD pathogenesis in diabetes.

The crucial role of LPL regulators in EAT metabolism, and their potential effect in the increase of EAT volume in CAD and DM2, turn them into interesting targets for new therapeutic agents.

4.1. Conclusion

This is the first time, to our knowledge, that LPL activity is reported in EAT, together with ANGPTL4, GPIHBP1 and PPARγ expression. The increase in LPL activity in diabetes, with no changes in its expression, suggests that the enzyme regulation would be mainly post-translational. The higher EAT LPL activity in DM2 could be partly responsible for the increase in EAT volume reported in these patients, and ANGPTL4 and GPIHBP1, as regulators of the enzyme, would be playing a crucial role in EAT metabolism. The fatty acids supply to EAT would partly come from circulating VLDL.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Financial support

This work was supported by the Agencia Nacional de Promoción Científica y Tecnológica (Grant number: PICT 0920-2016), the Universidad de Buenos Aires (Grant number: UBACYT 20020150-100042BA), the Sociedad Argentina de Diabetes (Grant number: SAD 2017–2018) and the Fundación Roemmers (Grant number: Roemmers 2016–2018).

Author contributions

Barchuk M has performed the experimental procedures, the statistical analysis and has contributed to the analysis of the results, manuscript writing and final preparation. Schreier L has contributed to the analysis of the results. Lopez G has performed the biochemical determinations. Cevey A and Goren N have performed the RT-qPCR

analysis. Rubio M and Baldi J have been responsible for the cardiovascular surgery and for obtaining EAT and SAT samples. Fernandez Tome MdelC has contributed to the assessment of VLDL characteristics. Miksztowicz V and Berg G have been responsible for the intellectual design, analysis of the results and manuscript writing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2019.06.915.

References

[1] Center for Disease Control, Prevention. National Diabetes Fact Sheet, (2011) http://www.cdc.gov/diabetes/pubs/pdf/ndfs_2011.pdf , Accessed date: 30 November 2013.

[2] P. Poirier, T.D. Giles, G.A. Bray, Y. Hong, J.S. Stern, et al., Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American heart association scientific statement on obesity and heart disease from the obesity committee of the council on nutrition, physical activity, and metabolism, Circulation 113 (6) (2006) 898–918.

[3] G. Iacobellis, A.E. Malavazos, Pericardial adipose tissue, atherosclerosis, and cardiovascular disease risk factors: the Jackson Heart Study: comment on Liu et al, Diabetes Care 33 (9) (2010) e127.

[4] T. Mazurek, L. Zhang, A. Zalewski, J.D. Mannion, J.T. Diehl, et al., Human epicardial adipose tissue is a source of inflammatory mediators, Circulation 108 (2003) 2460–2466.

[5] G. Iacobellis, D. Pistilli, M. Gucciardo, F. Leonetti, F. Miraldi, et al., Adiponectin expression in human epicardial adipose tissue in vivo is lower in patients with coronary artery disease, Cytokine 29 (2005) 251–255.

[6] V. Miksztowicz, C. Morales, M. Barchuk, G. López, R. Póveda, R. Gelpi, et al., Metalloproteinase 2 and 9 activity increase in epicardial adipose tissue of patients with coronary artery disease, Curr. Vasc. Pharmacol. 15 (2) (2017) 135–143.

[7] B. Gaborit, C. Sengenes, P. Ancel, A. Jacquier, A. Dutour, Role of epicardial adipose tissue in health and disease: a matter of fat? Comp. Physiol. 7 (3) (2017) 1051–1082.

[8] T. Seker, C. Turkoglu, H. Harbalıoglu, M. Gur, The impact of diabetes on the association between epicardial fat thickness and extent and complexity of coronary artery disease in patients with non-ST elevation myocardial infarction, Kardiol. Pol. 75 (11) (2017) 1177–1184.

[9] E.M. Groves, A.S. Erande, C. Le, J. Salcedo, K.C. Hoang, S. Kumar, et al., Comparison of epicardial adipose tissue volume and coronary artery disease severity in asymptomatic adults with versus without diabetes mellitus, Am. J. Cardiol. 114 (5) (2014) 686–691.

[10] J. Kang, Y.C. Kim, J.J. Park, S. Kim, S.H. Kang, et al., Increased epicardial adipose tissue thickness is a predictor of new-onset diabetes mellitus in patients with coronary artery disease treated with high-intensity statins, Cardiovasc. Diabetol. 17 (1) (2018) 10.

[11] M.M. Lima-Martínez, L. Colmenares, Y. Campanelli, M. Paoli, M. Rodney, et al., Epicardial adipose tissue thickness and type 2 diabetes risk according to the FINDRISC modified for Latin America, Clín. Invest. Arterioscler. (2018), https://doi.org/10.1016/j.arteri.2018.06.002.

[12] V. Camarena, D. Sant, M. Mohseni, T. Salerno, M.L. Zaleski, G. Wang, et al., Novel atherogenic pathways from the differential transcriptome analysis of diabetic epicardial adipose tissue, Nutr. Metabol. Cardiovasc. Dis. 27 (8) (2017) 739–750.

[13] V. Miksztowicz, L. Schreier, M. McCoy, D. Lucero, E. Fassio, et al., Role of SN1 lipases on plasma lipids in metabolic syndrome and obesity, Arterioscler. Thromb. Vasc. Biol. 34 (3) (2014) 669–675.

[14] S.G. Young, B.S. Davies, C.V. Voss, P. Gin, M.M. Weinstein, et al., GPIHBP1, an endothelial cell transporter for lipoprotein lipase, J. Lipid Res. 52 (11) (2011) 1869–1884.

[15] S. Kersten, Physiological regulation of lipoprotein lipase, Biochim. Biophys. Acta 1841 (7) (2014) 919–933.

[16] T. Ruge, V. Sukonina, O. Kroupa, E. Makoveichuk, M. Lundgren, et al., Effects of hyperinsulinemia on lipoprotein lipase, angiopoietin-like protein 4, and glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 in subjects with and without type 2 diabetes mellitus, Metabolism 61 (5) (2012) 652–660.

[17] I. Jaffer, M. Riederer, P. Shah, P. Peters, F. Quehenberger, et al., Expression of fat mobilizing genes in human epicardial adipose tissue, Atherosclerosis 220 (1) (2012) 122–127.

[18] American Diabetes Association, Classification and diagnosis of diabetes: standards of medical care in diabetes-2018, Diabetes Care 41 (Suppl 1) (2018) S13–S27.

[19] D.R. Matthews, J.P. Hosker, A.S. Rudenski, B.A. Naylor, D.F. Treacher, et al., Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 28 (1985) 412–419.

[20] V.N. Schumaker, D.L. Puppione, Sequential flotation ultracentrifugation, Methods Enzymol. 128 (1986) 155–170.

[21] C.H. Fiske, Y. Subbarow, The colorimetric determination of phosphorus, J. Biol. Chem. 66 (1925) 375–400.

[22] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[23] P. Nilsson-Ehle, R. Ekman, Rapid, simple and specific assay for lipoprotein lipase and hepatic lipase, Artery 3 (3) (1977) 194–209.

[24] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative C(T) method, Nat. Protoc. 3 (6) (2014) 1101–1108.

[25] A.M. Noyes, K. Dua, R. Devadoss, L. Chhabra, Cardiac adipose tissue and its relationship to diabetes mellitus and cardiovascular disease, World J. Diabetes 5 (6) (2014) 868–876.

[26] A.P. Chiu, F. Wang, N. Lal, Y. Wang, D. Zhang, et al., Endothelial cells respond to hyperglycemia by increasing the LPL transporter GPIHBP1, Am. J. Physiol. Endocrinol. Metab. 306 (11) (2014) E1274–E1283.

[27] M. Barchuk, V. Miksztowicz, V. Zago, A. Cevey, G. López, et al., Endothelial lipase is an alternative pathway for fatty acid release from lipoproteins: evidence from a high fat diet model of obesity in rats, Lipids 53 (10) (2018) 993–1003.

[28] A.P. Beigneux, B.S. Davies, P. Gin, M.M. Weinstein, E. Farber, et al., Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons, Cell Metabol. 5 (2007) 279–291.

[29] G.M. Dallinga-Thie, R. Franssen, H.L. Mooij, M.E. Visser, H.C. Hassing, et al., The metabolism of triglyceride-rich lipoproteins revisited: new players, new insight, Atherosclerosis 211 (2010) 1–8.

[30] R.P. Surendran, S.D. Udayyapan, M. Clemente-Postigo, S.R. Havik, et al., Decreased GPIHBP1 protein levels in visceral adipose tissue partly underlie the hypertriglyceridemic phenotype in insulin resistance, PLoS One 13 (11) (2018) e0205858.

[31] W. Dijk, A.P. Beigneux, M. Larsson, A. Bensadoun, S.G. Young, et al., Angiopoietin-like 4 (ANGPTL4) promotes intracellular degradation of lipoprotein lipase in adipocytes, J. Lipid Res. 58 (12) (2016) 7250–7257.

[32] A.P. Beigneux, C.M. Allan, N.P. Sandoval, G.W. Cho, P.J. Heizer, et al., Lipoprotein lipase is active as a monomer, Proc. Natl. Acad. Sci. U. S. A. (2019) 201900983, https://doi.org/10.1073/pnas.1900983116 ([Epub ahead of print]).

[33] A. Cinkajzlová, M. Mráz, Z. Lacinová, J. Kloučková, P. Kaválková, et al., Angiopoietin-like protein 3 and 4 in obesity, type 2 diabetes mellitus, and malnutrition: the effect of weight reduction and realimentation, Nutr. Diabetes 8 (1) (2018) 21.

[34] S. Romeo, L.A. Pennacchio, Y. Fu, E. Boerwinkle, A. Tybjaerg-Hansen, et al., Population-based resequencing of ANGPTL4 uncovers variations that reduce triglycerides and increase HDL, Nat. Genet. 39 (2007) 513–516.

[35] S. Romeo, W. Yin, J. Kozlitina, L.A. Pennacchio, E. Boerwinkle, et al., Rare loss-of-function mutations in ANGPTL family members contribute to plasma triglyceride levels in humans, J. Clin. Investig. 119 (2009) 70–79.

[36] P.J. Talmud, M. Smart, E. Presswood, J.A. Cooper, V. Nicaud, et al., ANGPTL4 E40K and T266M: effects on plasma triglyceride and HDL levels, postprandial responses, and CHD risk, Arterioscler. Thromb. Vasc. Biol. 28 (2008) 2319–2325.

[37] W. Dijk, S. Schutte, E.O. Aarts, I.M.C. Janssen, L. Afman, et al., Regulation of angiopoietin-like 4 and lipoprotein lipase in human adipose tissue, J Clin Lipidol 12 (3) (2018) 773–783.

[38] P.G. Blanchard, V. Turcotte, M. Côté, Y. Gélinas, S. Nilsson, et al., Peroxisome proliferator-activated receptor γ activation favours selective subcutaneous lipid deposition by coordinately regulating lipoprotein lipase modulators, fatty acid transporters and lipogenic enzymes, Acta Physiol. 217 (3) (2016) 227–239.

[39] B.S. Davies, H. Waki, A.P. Beigneux, E. Farber, M.M. Weinstein, et al., The expression of GPIHBP1, an endothelial cell binding site for lipoprotein lipase and chylomicrons, is induced by peroxisome proliferator-activated receptor-gamma, Mol. Endocrinol. 22 (2008) 2496–2504.

[40] G. Iacobellis, G. Barbaro, Epicardial adipose tissue feeding and overfeeding the heart, Nutrition 59 (2019) 1–6.

[41] A.P. Chiu, D. Bierende, N. Lal, F. Wang, A. Wan, I. Vlodavsky, Dual effects of hyperglycemia on endothelial cells and cardiomyocytes to enhance coronary LPL activity, Am. J. Physiol. Heart Circ. Physiol. 314 (1) (2018) H82–H94.

[42] B. Lu, A. Moser, J.K. Shigenaga, C. Grunfeld, K.R. Feingold, The acute phase response stimulates the expression of angiopoietin like protein 4, Biochem. Biophys. Res. Commun. 391 (4) (2010) 1737–1741.

[43] Lucero D, Zago V, López GH, Cacciagiú L, López GI, et al. Predominance of large VLDL particles in metabolic syndrome, detected by size exclusion liquid chromatography. Clin. Biochem.;45(4–5):293-297.

[44] L. Nasarre, O. Juan-Babot, P. Gastelurrutia, A. Llucia-Valldeperas, L. Badimon, et al., Low density lipoprotein receptor-related protein 1 is upregulated in epicardial fat from type 2 diabetes mellitus patients and correlates with glucose and triglyceride plasma levels, Acta Diabetol. 51 (1) (2014) 23–30.

[45] D. Chen, Y. Wang, K. Wu, X. Wang, Dual effects of metformin on adipogenic differentiation of 3t3-L1 preadipocyte in AMPK-dependent and independent manners, Int. J. Mol. Sci. 19 (6) (2018) pii: E1547.

[46] H. Mirmiranpour, M. Mousavizadeh, S. Noshad, M. Ghavami, M. Ebadi, et al., Comparative effects of pioglitazone and metformin on oxidative stress markers in newly diagnosed type 2 diabetes patients: a randomized clinical trial, J. Diabet. Complicat. 27 (5) (2013) 501–507.

[47] G. Iacobellis, M. Mohseni, S.D. Bianco, P.K. Banga, Liraglutide causes large and rapid epicardial fat reduction, Obesity 25 (2) (2017) 311–316.