Overexpression of scavenger receptor and infiltration of macrophage in epicardial adipose tissue of patients with ischemic heart disease and diabetes

Concepción Santiago-Fernández1,2, Luis M. Pérez-Belmonte3,4*, Mercedes Millán-Gómez3,4, Inmaculada Moreno-Santos3,4, Fernando Carrasco-Chinchilla3,4, Amalio Ruiz-Salas3,4, Luis Morcillo-Hidalgo3,4, José M. Melero3,4, Lourdes Garrido-Sánchez1,2† and Manuel Jiménez-Navarro3,4†

Abstract

Background: Oxidized low-density lipoproteins and scavenger receptors (SRs) play an important role in the formation and development of atherosclerotic plaques. However, little is known about their presence in epicardial adipose tissue (EAT). The objective of the study was to evaluate the mRNA expression of different SRs in EAT of patients with ischemic heart disease (IHD), stratifying by diabetes status and its association with clinical and biochemical variables.

Methods: We analyzed the mRNA expression of SRs (LOX-1, MSR1, CXCL16, CD36 and CL-P1) and macrophage markers (CD68, CD11c and CD206) in EAT from 45 patients with IHD (23 with type 2 diabetes mellitus (T2DM) and 22 without T2DM) and 23 controls without IHD or T2DM.

Results: LOX-1, CL-P1, CD68 and CD11c mRNA expression were significantly higher in diabetic patients with IHD when compared with those without T2DM and control patients. MSR1, CXCL16, CD36 and CD206 showed no significant differences. In IHD patients, LOX-1 (OR 2.9; 95% CI 1.6–6.7; P = 0.019) and CD68 mRNA expression (OR 1.7; 95% CI 0.98–4.5; P = 0.049) were identified as independent risk factors associated with T2DM. Glucose and glycated hemoglobin were also shown to be risk factors.

Conclusions: SRs mRNA expression is found in EAT. LOX-1 and CD68 and were higher in IHD patients with T2DM and were identified as a cardiovascular risk factor of T2DM. This study suggests the importance of EAT in coronary atherosclerosis among patients with T2DM.

Keywords: Scavenger receptors, Oxidized low-density lipoprotein, Epicardial adipose tissue, Diabetes mellitus, Ischemic heart disease

*Correspondence: luismiguelpb1984@gmail.com; lourgarrido@gmail.com
†Lourdes Garrido-Sánchez and Manuel Jiménez-Navarro contributed equally to this work and shared the last authorship
1 Department of Endocrinology and Nutrition, Virgen de la Victoria Hospital (IBIMA), Malaga University, Campus de Teatinos s/n, 29010 Malaga, Spain
2 Unidad de Gestión Clínica Área del Corazón, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Universitario Virgen de la Victoria, Universidad de Málaga (UMA), Campus Universitario de Teatinos, s/n, Malaga, Spain
Full list of author information is available at the end of the article

© The Author(s) 2019. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background

Ischemic heart disease (IHD) is a major cause of death and disability in developed countries. Although IHD mortality rates worldwide have declined over the last decades, it persists as responsible for one-third or more of all deaths in adult individuals [1, 2]. Multiple cardiovascular risk factors contribute to the pathogenesis of atherosclerosis [3]. Different strategies have been proposed for improving prognosis (mainly death and hospitalizations) such as percutaneous coronary revascularization, coronary artery bypass surgery and cardiac rehabilitation [4].

In recent years, epicardial adipose tissue (EAT) has been proposed as playing a relevant role in the pathophysiology of IHD [5–8]. EAT is located between the myocardium and the serous layer of the pericardium, and in close proximity to the coronary arteries [9]. It was reported that EAT thickness is an indicator of cardiovascular risk [10]. In physiological conditions, EAT participates in the protection of the myocardium and the coronary vessel, maintaining the energy balance. However, dysfunctional EAT has been implicated in the progression and more aggressive course of IHD. One of these pathological conditions which might alter the normal functionality of EAT is the presence of type 2 diabetes mellitus (T2DM) [11, 12]. An altered EAT is able locally to produce reactive oxygen species, cytokines and chemokines which may create a local toxic and proinflammatory environment [13–15]. The inflammation of EAT has been linked to IHD pathophysiology, which can be reflected by increased of macrophage infiltration [16, 17]. In this state, EAT shows a high infiltration of leukocytes [18], specifically T lymphocytes and macrophages [18, 19] and inflammatory cytokines [20].

It is well known that oxidative stress plays an important role in the genesis of T2DM. The increase in systemic oxidative stress seems to be an important mechanism leading to the increase in lipid peroxidation and the oxidative modification of LDL [20]. Oxidized low-density lipoproteins (OxLDLs) play an important role in the formation and development of atherosclerotic plaques and have been associated with most of the proatherogenic risk factors, including obesity, dyslipidemia, metabolic syndrome and T2DM [21–23]. OxLDLs are mainly removed from circulation by a family of membrane bound receptors, called scavenger receptors (SRs). Different classes of SRs, such as Lectin-like Oxidized LDL receptor-1 (LOX-1), CD36, Macrophage scavenger receptor 1 (MSR1), C–X–C motif Chemokine Ligand 16 (CXCL16) and Collectin Placenta 1 (CL-P1) have been identified in various cell types. The expression of these receptors depends largely on the cell type and cell activation, therefore the uptake and subsequent effect of OxLDLs may be different [24]. The presence of these receptors in adipose tissue could be mainly due to the presence of SR in macrophages. However, several studies have shown their presence in adipocytes [25] which could play a role in the metabolism of circulating lipoproteins, including OxLDLs [24, 26, 27].

Based on the evidence mentioned above, the aim of our study was to evaluate the expression of different SRs (LOX-1, MSR1, CD36, CXCL16, CL-P1) and the measure of macrophage infiltration (Cluster Differentiation 68 (CD68), CD11c and CD206 in EAT in patients with IHD, stratifying by T2DM status. We hypothesized that the mRNA expression of SRs and the infiltration of macrophages in EAT would be different according to the presence of T2DM. We also assessed the possible association between SR expression and clinical and biochemical variables.

Methods

Patients

We included 45 patients with IHD who underwent coronary artery bypass surgery (IHD group) and 23 patients without IHD who underwent aortic and/or mitral valve replacement surgery (control group) due to the first way to obtain EAT in patients without IHD. The group with patients with IHD was divided according to T2DM status: those with T2DM (n = 23) (IHD-T2DM group) and those without T2DM (n = 22) (IHD-NoT2DM group). The IHD group was defined by the presence of at least one coronary stenosis ≥ 50% of luminal diameter by coronary angiogram. The control group had chronic valve heart disease with or without stenosis less than 50% in any vessel requiring valve replacement but not IHD and without T2DM. T2DM was defined as having a history of diabetes diagnosed and/or treated with medication, fasting blood glucose ≥ 126 mg/dL and/or glycated hemoglobin (HbA1c) ≥ 6.5. Diabetic treatment of IHD patients with T2DM was: only diet (n = 6, 26.1%), oral anti-diabetic (n = 12, 52.1%), oral anti-diabetic and insulin (n = 4, 17.4%) and only insulin (n = 1, 4.3%). No patient was taking thiazolidinediones. In the IHD-T2DM patient group, the duration of diabetes was 6.7 ± 2.2 years. Dyslipidemia was defined as having a history of diagnosed and/or treated with medication for elevated triglycerides, low HDL-cholesterol or high LDL-cholesterol. The presence of greater than or equal to 50% luminal diameter stenosis in at least one major epicardial artery by coronary angiogram defined the IHD. Single vessel and multi-vessel disease were defined as the presence of this stenosis in one major epicardial artery and in two or more major epicardial arteries, respectively. Calculation of the Gensini score was initiated by giving a severity score to each coronary stenosis as follows: 1 point.
for ≤ 25% narrowing, 2 points for 26 to 50% narrowing, 4 points for 51 to 75% narrowing, 8 points for 76 to 90% narrowing, 16 points for 91 to 99% narrowing, and 32 points for total occlusion. Thereafter, each lesion score
narrowing, 16 points for 91 to 99% narrowing, and 3 points for 51 to 75% narrowing, 8 points for 76 to 90% narrowing.

Finally, the Gensini score was calculated by summation of the individual coronary segment scores [28]. Patients with coronary disease with lesions that required revascularization surgery and those patients in the control group who had chronic valve heart disease without IHD or DM were included in the study. Moreover, the decision that these patients were included in the study was taken in the clinical session by cardiac surgeons and cardiology experts. Patients with acute inflammatory disease, severe infectious diseases and/or cancer and women who were taking hormone replacement were excluded from the study. All patients gave written informed consent, and the study protocol was approved by the local Clinical Research Ethics Committee and was carried out in accordance with the Declaration of Helsinki.

**Laboratory measurements**

Peripheral venous blood was drawn into pyrogen free tubes with or without EDTA as an anticoagulant on the morning of surgery. For serum, the tubes were left at room temperature for 20 min and then centrifuged at 1500g for 10 min at 4 °C. Fasting glucose, glycated hemoglobin (HbA1c), basal insulin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, apoA-1, apoB-100 and C-reactive protein (CRP) were measured in a Dimension analyzer (Dade Behring Inc., Deerfield, IL) in the hospital laboratory. sLRP1 concentration was measured using commercially available enzyme-linked immunosorbent assay (Abbexa, Cambridge, UK) according to the manufacturer’s recommendation. Homeostasis model assessment for insulin resistance (HOMA-IR) score was calculated from fasting insulin and glucose:

\[
\text{HOMA-IR} = \frac{\text{fasting insulin (μIU/mL)} \times \text{fasting glucose (mmol/L)}}{22.5}
\]

The concentration of OxLDL was measured by a solid phase two site ELISA (Mercodia Developing Diagnostic, Uppsala, SWEDEN). The intra and inter-assay coefficients of variation were 6.4 and 4.7%, respectively.

**Biological material**

EAT biopsy samples (average 0.2 to 0.5 g) were obtained with a precise surgical technique during the heart surgery, near the proximal right coronary artery and located between the myocardium and the visceral pericardium. Pericardial adipose tissue is located on the external surface of the parietal pericardium and is vascularized from non-coronary sources [29]. The sample was collected approximately 1 h after the beginning of general anesthesia. All the tissues were washed in physiological saline, immediately frozen in liquid nitrogen and maintained at −80 °C until RNA analysis.

**RNA extraction and real time quantitative transcription polymerase chain reaction**

Total RNA from frozen human EAT was isolated using RNeasy Lipid Tissue Mini Kit (Qiagen, GmbH, Germany) as previously described [30–32]. Total RNA was reverse transcribed using random hexamers as primers and transcription reverse transcriptase (Roche, Mannheim, Germany). Gene expression was assessed by real time PCR using an Applied Biosystems 7500 Fast real time polymerase chain reaction system (Applied Biosystems, Darmstadt, Germany). Reactions were carried out in duplicate for all genes using specific TaqMan® Gene Expression Assays: macrophage scavenger receptor 1 (MSRI) (Hs00234007_m1, RefSeq. NM_002445.3, NM_138715.2, NM_138716.2), chemokine (C–X–C motif) ligand 16 (CXCL16) (Hs00222859_m1, RefSeq. NM_01100812.1, NM_022059.2), oxidized low-density lipoprotein (lectin-like) receptor 1 (LOX-1) (Hs01552593_m1, RefSeq. NM_001172632.1, NM_001172633.1, NM_002543.3), collectin sub-family member 12 (CL-PI) (Hs00560477_m1, RefSeq. NM_130386.2), CD68 (Hs00154355_m1, RefSeq. NM_001040059.1, NM_001251.2), integrin subunit alpha X (CD11c) (Hs00174217_m1, RefSeq. NM_000887.4, NM_001286375.1), mannose receptor, C type 1 (CD206) (Hs00267207_m1, RefSeq. NM_00267207_m1), CD36 (Hs01567185_m1, RefSeq. NM_000072.3, NM_001001547.2, NM_001001548.2, NM_001127443.1, NM_001127444.1, NM_001289909.1, NM_001289911.1) and LDL receptor related protein 1 (LRP1) (Hs00233856_m1, RefSeq. NM_002332.2). During PCR, the threshold value for all genes studied was 0.1. The Ct value for each sample was normalized by constitutively expressed cyclophilin A signals (PPIA) (4326316E, RefSeq. NM_021130.3). SDS software 2.3 and RQ Manager 1.2 (Applied Biosystems, Foster City, CA) were used to analyze the results with the comparative Ct method
(2−ΔΔCt). All data were expressed as an n-fold difference relative to the calibrator.

Statistical analysis
Statistical analyses were performed with SPSS for Windows version 15 (SPSS Inc. Chicago, IL, USA). The normality of continuous variables was checked by means of the Kolmogorov–Smirnov test. Continuous variables are summarized as mean ± standard deviation, and as mean ± SEM in figures. Discrete variables are summarized as frequencies (percentages). The comparison between the results of the different groups was made with the Chi square test and Fischer exact test for categorical data, or with the analysis of variance (ANOVA) and Kruskal–Wallis for continuous variables. Logistic regression models were used in order to identify independent factors (odds ratio [OR]; 95% confidence interval) for IHD in patients with T2DM associated with mRNA expression of SRs, as well as to control for confounding factors. The inclusion of 7 patients in each group for an α of 0.05 (assuming a difference of 0.22 of CD36 mRNA expression, a standard deviation of 0.14 and a power of 0.80) has the power to detect a significance difference of 95%. Values were considered to be statistically significant when P < 0.05.

Results
Clinical and biochemical characteristics of the patients
A total of 68 patients were included in our study. Among the 45 patients of the IHD Group, 23 (51.1%) had T2DM (IHD-T2DM group). Table 1 summarizes the anthropometric, clinical and biochemical characteristics of the IHD groups and controls. No significant differences were found between the groups regarding age, gender, cardiovascular risk factors and left ventricular ejection fraction. However, IHD-T2DM patients showed significantly higher levels of fasting glucose, HbA1c, insulin, HOMA-IR score and C-reactive protein than IHD-NoT2DM and controls. The levels of oxLDL and LRP1 were significantly higher for LOX-1, CL-P1, CD68 and CD11c in EAT when compared with IHD-NoT2DM patients (P < 0.01) and control patients (P < 0.01) (Fig. 1a, b, e and g, respectively). However, mRNA expression of MSR1, CXCL16, CD36, CD206 and LRP1 showed no significant differences (Fig. 1c, d, f, h and i, respectively). The mRNA expressions of SRs were similar in IHD-NoT2DM and control patients (Fig. 1).

With respect to the distribution of M1 and M2 macrophages, significant differences were found in CD11c/CD68 ratio (M1 macrophages) between IHD-T2DM and control group, showing a higher level those IHD-DM patients (Fig. 2a). No significant differences were found in the CD206/CD68 ratio (M2 macrophages) (Fig. 2b).

Associations in EAT between the mRNA expression of SRs and the presence of T2DM in patients with IHD
The factors associated with the presence of T2DM in patients with IHD in a logistic regression model was the level of LOX-1 and CD68 mRNA expression in EAT. These receptors were identified as independent risk factors of suffering T2DM. Glucose and HbA1c were also shown to be risk factors. This regression was adjusted for sex, age, BMI, statins, FRS and CL-P1 mRNA expression (Table 2). No significant association was found between LOX-1 and T2DM duration.

Discussion
The main finding of our study was that the mRNA expression of SRs is expressed in EAT and this expression was significantly higher in IHD-T2DM when compared with IHD-NoT2DM patients (P < 0.01) and control patients (P < 0.01) (Fig. 1a, b, e and g, respectively). However, mRNA expression of MSR1, CXCL16, CD36, CD206 and LRP1 showed no significant differences (Fig. 1c, d, f, h and i, respectively). The mRNA expressions of SRs were similar in IHD-NoT2DM and control patients (Fig. 1).

With respect to the distribution of M1 and M2 macrophages, significant differences were found in CD11c/CD68 ratio (M1 macrophages) between IHD-T2DM and control group, showing a higher level those IHD-DM patients (Fig. 2a). No significant differences were found in the CD206/CD68 ratio (M2 macrophages) (Fig. 2b).

Associations in EAT between the mRNA expression of SRs and the presence of T2DM in patients with IHD
The factors associated with the presence of T2DM in patients with IHD in a logistic regression model was the level of LOX-1 and CD68 mRNA expression in EAT. These receptors were identified as independent risk factors of suffering T2DM. Glucose and HbA1c were also shown to be risk factors. This regression was adjusted for sex, age, BMI, statins, FRS and CL-P1 mRNA expression (Table 2). No significant association was found between LOX-1 and T2DM duration.

Discussion
The main finding of our study was that the mRNA expression of SRs is expressed in EAT and this expression was significantly higher for LOX-1 and CL-P1 in IHD-T2DM patients. On the other hand, we found that the infiltration of macrophages was enhanced in the EAT of IHD-T2DM patients, when compared with the IHD-NoT2DM and control group. In addition, the expression of LOX-1 and CD68, glucose and HbA1c levels were identified as risk factors of suffering T2DM. Glucose and HbA1c were also shown to be risk factors. This regression was adjusted for sex, age, BMI, statins, FRS and CL-P1 mRNA expression (Table 2). No significant association was found between LOX-1 and T2DM duration.
overexpressed [35]. In our study, we showed an overexpression of LOX-1 in EAT among patients with IHD and T2DM compared with IHD without T2DM and control group, suggesting that this adipose tissue which is anatomically in direct contact with the heart and coronary arteries, could be linked to coronary atherosclerosis. Our results showing an association between LOX-1 expression and T2DM and glucose agree with previous

| Variables                                      | IHD-T2DM (n = 23) | IHD-NoT2DM (n = 22) | Control (n = 23) | P value* | P value** | P value*** | P value
|------------------------------------------------|-------------------|--------------------|-----------------|----------|----------|-----------|----------
| Age (years)                                    | 64.2 ± 10.1       | 65 ± 10.8          | 62 ± 10         | 0.451    | 0.302    | 0.196     | 0.298
| Male gender                                    | 18 (78.3%)        | 18 (81.8%)         | 15 (65.2%)      | 0.530    | 0.257    | 0.257     | 0.467
| BMI (kg/m²)                                    | 28.6 ± 6.6        | 28.5 ± 4.5         | 27.8 ± 4.1      | 0.734    | 0.675    | 0.410     | 0.689
| Smoking                                        | 17 (73.9%)        | 16 (72.7%)         | 12 (52.2%)      | 0.545    | 0.352    | 0.373     | 0.498
| Obesity                                        | 13 (56.5%)        | 11 (50%)           | 10 (43.4%)      | 0.362    | 0.197    | 0.456     | 0.241
| Hypertension                                   | 20 (86.9%)        | 18 (81.8%)         | 17 (74%)        | 0.314    | 0.176    | 0.422     | 0.217
| Dyslipidemia                                   | 19 (82.6%)        | 18 (81.8%)         | 16 (69.6%)      | 0.574    | 0.117    | 0.176     | 0.196
| Cerebrovascular disease                         | 2 (8.7%)          | 1 (4.5%)           | 2 (8.7%)        | 0.500    | 0.744    | 0.482     | 0.485
| Cardiovascular disease history                  | 23 (100%)         | 22 (100%)          | 2 (8.7%)        | 0.687    | 0.035    | 0.040     | 0.048
| Cardiovascular disease family history           | 6 (26.1%)         | 3 (13.6%)          | 1 (4.3%)        | 0.146    | 0.078    | 0.357     | 0.176
| Left ventricular ejection fraction (%)         | 55 ± 6            | 53 ± 6             | 52 ± 6          | 0.352    | 0.146    | 0.397     | 0.284
| Left ventricular ejection fraction ≤ 40%       | 5 (21.7%)         | 4 (18.2%)          | 4 (17.4%)       | 0.112    | 0.115    | 0.651     | 0.209
| Framingham score                               | 14.4 ± 8.1        | 16.8 ± 9.5         | 9.6 ± 5.8       | 0.546    | 0.112    | 0.048     | 0.093

| Medications, n (%)                              | 12 (52.2)         | 8 (36.4)           | 9 (39.1)        | 0.354    | 0.346    | 0.850     | 0.423
| ACEI/ARB                                       | 10 (43.4)         | 5 (22.7)           | 6 (26.1)        | 0.231    | 0.205    | 0.796     | 0.215
| Beta-blocker                                   | 14 (60.9)         | 8 (36.4)           | 15 (65.2)       | 0.129    | 0.922    | 0.076     | 0.095

| Biochemical data                               |                   |                    |                 |          |          |          |          |
| Glucose (mg/dL)                                 | 167 ± 31          | 100 ± 29           | 100 ± 29        | <0.001   | <0.001   | 0.919     | <0.001
| HbA1c (%)                                      | 7.9 ± 1           | 5.7 ± 0.5          | 5.5 ± 0.5       | <0.001   | <0.001   | 0.186     | <0.001
| Insulin (µIU/mL)                                | 196 ± 15          | 7 ± 4.4            | 9.4 ± 5         | 0.133    | 0.215    | 0.500     | 0.187
| HOMA-IR                                       | 103 ± 8.3         | 1.7 ± 1            | 2.5 ± 1.6       | 0.133    | 0.111    | 0.527     | 0.111
| Cholesterol (mg/dL)                             | 163 ± 31          | 157 ± 28           | 150 ± 29        | 0.641    | 0.108    | 0.158     | 0.199
| LDL cholesterol (mg/dL)                         | 101 ± 21          | 99 ± 19            | 103 ± 21        | 0.910    | 0.925    | 0.970     | 0.899
| HDL cholesterol (mg/dL)                         | 34 ± 6            | 41 ± 9             | 44 ± 9          | 0.033    | 0.001    | 0.427     | 0.004
| Triglycerides (mg/dL)                           | 189 ± 59          | 169 ± 41           | 148 ± 40        | 0.202    | 0.004    | 0.133     | 0.014
| ApoB-100/ApoA-1                                 | 0.523 ± 0.101     | 0.743 ± 0.230      | 0.757 ± 0.240   | 0.267    | 0.222    | 0.998     | 0.287
| OxLDL (units/L)                                | 63.4 ± 10         | 54.4 ± 5.3         | 51.0 ± 8.6      | 0.118    | 0.033    | 0.472     | 0.081
| LRP1 (ng/mL)                                   | 0.925 ± 0.438     | 0.677 ± 0.493      | 0.789 ± 0.267   | 0.093    | 0.036    | 0.257     | 0.067
| CRP (mg/dL)                                    | 71.6 ± 42         | 27.3 ± 19          | 19.2 ± 22       | 0.138    | 0.056    | 0.354     | 0.104

| Characteristics of coronary disease            |                   |                    |                 |          |          |          |          |
| Multivessel coronary disease                    | 19 (82.6%)        | 13 (59.1%)         |                 | 0.01     |          |          |          |
| > 50% stenosis LMA                              | 16 (69.6%)        | 10 (45.5%)         |                 | 0.01     |          |          |          |
| > 50% stenosis ADA                              | 22 (95.7%)        | 16 (72.7%)         |                 | 0.01     |          |          |          |
| > 50% stenosis CA                               | 18 (78.3%)        | 13 (59.1%)         |                 | 0.01     |          |          |          |
| > 50% stenosis RCA                              | 19 (85.6%)        | 14 (63.6%)         |                 | 0.01     |          |          |          |
| Gensini score                                   | 45 ± 14           | 34 ± 12            |                 | <0.001   |          |          |          |

Values are shown as mean ± SD and frequencies (percentages)

ADA, anterior descending artery; CA, circumflex artery; IHD-DM, ischemic heart disease-diabetes mellitus; IHD-NDM, ischemic heart disease-non diabetes mellitus; CRP, C-reactive protein; BMI, body mass index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance IU/L, international units/liter; kg/m², kilogram/square metre; LDL, low-density lipoprotein; LMA, left main artery; mg/dL, milligram/deciliter; mmol/L, millimol/liter; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; OxLDL, oxidized low-density lipoprotein; LRP1, low density lipoprotein receptor-related protein 1; RCA, right coronary artery

P value: overall comparison for all groups. * P value: IHD-T2DM vs. IHD-NoT2DM; ** P value: IHD-T2DM vs. control group; *** P value: IHD-NoT2DM vs. control group
studies in which \( \text{LOX-1} \) expression was induced by high glucose levels, which seems to be NADPH oxidase-dependent [37]. In line with our findings, the overexpression of \( \text{LOX-1} \) in several tissues has also been related with the development and progression of T2DM and its cardiovascular complications [38, 39]. The role of \( \text{LOX-1} \) in myocardial ischemia has been shown in some reports. Li et al. [40] showed up-regulated \( \text{LOX-1} \) levels in the heart after a short period of coronary artery occlusion, associating with markers of inflammation, oxidative stress, and apoptosis. Similarly, Lu et al. [41] studied the modulation of myocardial damage and heart function induced by permanent coronary occlusion and found that the \( \text{LOX-1} \) gene deletion improved survival in mice. In addition, \( \text{LOX-1} \) has been also implicated in the collagen deposition after myocardial ischemia, favoring cardiac remodeling [35, 41]. Its gene deletion importantly reduced the process of cardiac remodeling and scar formation, preserving ventricular ejection fraction in mice [41].

With respect to the macrophage infiltration, our work has shown that mRNA expression of \( \text{CD68} \) was increased in EAT in patients with IHD and T2DM when compared with patients without T2DM and control group. In this sense, there are studies that have shown an increase in macrophage infiltration in the EAT of CAD patients, reflected by increased \( \text{CD68} \) macrophage infiltration [16, 17]. In recent years, several studies have indicated that insulin resistance and T2DM are associated with the inflammation of adipose tissue [42–44]. However, limited studies have focused on the inflammatory profile of EAT and its possible involvement with atherosclerosis [6, 11, 43]. In harmony with our findings, Bambace et al., determined concomitantly mRNA expression levels of \( \text{CD68} \) in both subcutaneous and epicardial adipose tissue.
in male patients with and without T2DM and observed higher CD68 gene expression levels in both tissue types in diabetic patients than in those without T2DM [43]. Moreover, when we study the distribution of macrophage subtypes, our study shows that only CD11c and CD11c/CD68 ratio (M1-macrophage phenotypes), but not CD206, were more significantly overexpressed in IHD-T2DM patients when compared to IHD-NDM or control group. In agreement with our findings, Gurses et al. [16] observed a shift to pro-inflammatory M1-macrophage phenotype in EAT of patients with coronary artery disease (CAD) compared to the control group (without CAD), reflected by increased of CD11c, CD11c/CD68 and CD11c/CD206 ratios, but not CD206. According to our results, Hirata et al. [17] showed similar results, demonstrated that pro-inflammatory macrophages are more dominant in EAT when compared with and without CAD patients. Therefore, our findings suggest that infiltration of macrophages could also cause local inflammation in EAT and these cells could leak free fatty acids. Also, the increase of macrophage infiltration seen in the T2DM patients is consistent with the increase of SRs expression in EAT. Moreover, we have found that macrophage infiltration and these SRs are associated with T2DM independently of BMI, factor directly involved in the adipose tissue inflammation. Our findings would suggest that is not just obesity and BMI that explains this relationship. In this sense, in a recent study, Antonopoulos et al. [45] show that adipose inflammation may contribute to atherosclerosis.

Regarding the mRNA expression levels of CL-P1, we described for the first time that CL-P1 is expressed in EAT and its expression is significantly greater in IHD-T2DM than in those patients without T2DM and controls. This finding could be related to the function of this SR, mediating the uptake of Ox-LDL and collaborating with other SRs in coronary atherosclerosis. However, its involvement in atherosclerosis has not been clearly described. CL-P1 plays a key role in host defense [46]. In a recent report, CL-P1 has been proposed as inhibiting complement activation and host damage in order to protect self-tissues in acute phase responses [47]. Its expression has been shown in human and murine vascular endothelial layers but its proangiogenic role has not been specifically described [48].

MSR1 and CXCL16 were also expressed in EAT, showing higher expression levels in diabetic patients with IHD than in those without T2DM and controls, however, the differences were not significant. Although SRs were originally identified by their ability to recognize and to remove OxLDL, they are very versatile, with a large repertoire of functions such as the elimination of physiological and microbial substances, a critical role in the innate immunity, lipid transport and tissue homeostasis. This wide heterogeneity determines the implication of these receptors in the pathogenesis of different diseases [49] and could explain the differences in the expression levels of these receptors.

### Table 2: Factors associated with type 2 diabetes mellitus in patients with ischemic heart disease

| VARIABLE | OR (95% CI) | P value | B coefficient |
|----------|-------------|---------|---------------|
| Glucose  | 5.9 (2.4–9.0) | < 0.01 | 0.688 |
| HbA1c    | 4.0 (1.9–8.1) | < 0.01 | 0.621 |
| LOX-1mRNA (AU) | 2.9 (1.6–6.7) | 0.019 | 0.599 |
| CD68 mRNA (AU) | 1.7 (0.98–4.5) | 0.049 | 0.531 |

OR (95% CI) and B coefficient are shown. Logistic regression analysis for T2DM in patients with IHD

LOX-1: Lectin-like Oxidized LDL receptor-1; CD68, cluster of differentiation 68; HbA1c, glycated hemoglobin; OR, odds ratio; 95% CI, 95% confidence interval; AU, arbitrary units
Everyday increasing number of diabetic patients and CAD are managed in hospitals and the number will be epidemic in next years due mainly to increase life expectancy and levels of obesity [50]. Proposed strategies achieve a clinical improvement to a certain level [51, 52] and new clinical scenarios such as incomplete revascularization are described [53]. Knowing exactly mechanisms that underlie us importance of EAT can help us to develop new therapeutic strategies and to be able to improve the prognosis of diabetic patients with coronary disease.

We would like to acknowledge some limitations of this study, which is descriptive and no mechanistic insight is provided to e.g. explain the increase in SRs expression in EAT or to identify the cells which express SRs in EAT. Also, this is not a study entirely new. Earlier reports have already described recruitment of macrophages to EAT [18, 19], with an increase in T2DM patients [43]. Moreover, we recruited a relatively small number of patients; our data were from a single hospital and only small samples of EAT were collected from each patient, being insufficient for a thorough analysis and correlation between mRNA expression and protein. Due to the small amount of EAT sample obtained from each patient, we could not measure CD31, as a marker of endothelial cells, and SCARB1 or ABCA1, involved in the regulation of cholesterol accumulation. Also, another limitation of the study is that we did not perform any specific cardiac test, out of our routine clinical practice, to measure the volume of EAT. EAT measurement requires experts trained specifically in cardiac imagination to obtain valuable data. However, our study was carried out using a well-designed protocol and well established methods. Finally, our study reveals an association, however not a clear causal relationship. The hypothesis that the SRs mRNA expression in EAT is different according to the presence of diabetes and that it could be involved in the pathophysiology of coronary atherosclerosis would need to be confirmed in further research. Therapeutic targeting to EAT regarding the SRs is one choice and this discovery-type of study by the authors would really need repeated investigations using other independent sample sets.

Conclusions
Our data revealed a predominantly inflammatory profile in EAT in diabetic patients with IHD in comparison with those without DM and controls, showing the implication of LOX-1 as the main SR expressed in EAT and infiltration of macrophage.

SR genes are expressed in EAT. LOX-1 and CD68 were higher in ischemic heart disease with T2DM than in those patients without T2DM and control patients, and were associated as a cardiovascular risk factor of ischemic heart disease and the severity of CAD, suggesting the importance of EAT in the coronary atherosclerosis among patients with T2DM.

Authors’ contributions
Design and coordination of the study: MJN, LMPB and LGS. Selection of subject: MJN, LMPB, FCC, ARS, LMH, JMM and LGS. Performed the experiments: CSG, MMG, LMPB and LGS. Contributed reagents/materials/analysis tools: MJN. Wrote the paper: MJN, LMPB and LGS. All authors helped to write the manuscript. All authors read and approved the final manuscript.

Author details
1 Department of Endocrinology and Nutrition, Virgen de la Victoria Hospital (IBIMA), Malaga University, Campus de Teatinos s/n, 29010 Malaga, Spain. 2 Centro de Investigación Biomédica en Red de la Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Malaga, Spain. 3 Unidad de Gestión Clínica Área del Corazón, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Universitario Virgen de la Victoria, Universidad de Málaga (UMA), Campus Universitario de Teatinos, s/n, Malaga, Spain. 4 Centro de Investigación Biomédica en Red Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Malaga, Spain.

Acknowledgements
The authors thank the cardiac surgeons from the Department of heart surgery (Virgen de la Victoria Hospital, Malaga) for their contribution in collecting samples. Lourdes Garrido-Sanchez was the recipient of the Nicolas Monardes Program from the “Servicio Andaluz de Salud, Junta de Andalucía” Spain (C-0028-2018) and of the Miguel Servet tipo II contract from ISCIII (CPI118/00303). C. Santiago-Fernandez is supported by a fellowship from ISCIII (“PFIS” program, F116-00241).

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets generated and/or analysed during the current study are not publicly available due patients privacy could be compromised, but are available from the corresponding author on reasonable request.

Consent for publication
Not applicable.

Ethics approval and consent to participate
All patients gave written informed consent, and the study protocol was approved by the local Clinical Research Ethics Committee and was carried out in accordance with the Declaration of Helsinki.

Funding
This work was supported by Grants from the Spanish Ministry of Health (FIS) (PI13/02542, PI11/01661) and Centro de Investigación Biomédica en Red Enfermedades Cardiovasculares (CIBERCV) (CB16/1/00368), Instituto de Salud Carlos III co-funded by the Fondo Europeo de Desarrollo Regional (FEDER).

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 17 November 2018   Accepted: 11 March 2019
Published online: 20 March 2019

References
1. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J. 2014;35:2950–9.
2. Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, et al. Heart disease and stroke statistics—2017 update: a report from the American Heart Association. Circulation. 2017;135:416–63.

3. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011;473:317–28.

4. Jiménez-Navarro MF, López-Jiménez F, Pérez-Belmonte LM, Llompart R, Díaz-Meleán C, Rodríguez-Escudero JP, et al. Benefits of cardiac rehabilitation on cardiovascular outcomes in patients with diabetes mellitus after percutaneous coronary intervention. J Am Heart Assoc. 2017;6:e006404. https://doi.org/10.1161/jaha.117.006404.

5. Albuquerque RN, Somers VK, Blume G, Miranda W, Korenfeld Y, Calvin AD, et al. Usefulness of epicardial adipose tissue as predictor of cardiovascular events in patients with coronary artery disease. Am J Cardiol. 2012;110:100–5.

6. Pérez-Belmonte LM, Moreno-Santos I, Cabrera-Bueno F, Sánchez-Espin G, Castellano D, Such M, et al. Expression of sterol regulatory element-binding proteins in epicardial adipose tissue in patients with coronary artery disease and diabetes mellitus: preliminary study. Int J Med Sci. 2017;14:268–74.

7. Franssens BT, Nathoe HM, Visseren FL, van der Graaf Y, Leiner T, SMART Study Group. Relation of epicardial adipose tissue radiodensity to coronary artery calcium on cardiac computed tomography in patients at high risk for cardiovascular disease. Am J Cardiol. 2017;119:1359–65.

8. Chen WJ, Danad I, Raijmakers PG, Halbmeijer R, Harms HJ, Lammertsma AA, et al. Effect of type 2 diabetes mellitus on epicardial adipose tissue volume and coronary vasomotor function. Am J Cardiol. 2014;113:90–7.

9. Iozzo P. Myocardial, perivascular, and epicardial fat. Diabetes Care. 2011;34:371–9.

10. Iacobellis G, Bianco AC. Epicardial adipose tissue: emerging physiological, pathophysiological, and clinical features. Trends Endocrinol Metab. 2012;23:450–7.

11. Malavazos AE, Corsi MM, Ermetici F, Coman C, Sardanelli F, Rossi A, et al. Interleukin-15 and soluble interleukin-15 receptor alpha in coronary artery disease: an autopsy study protocol. JMIR Res Protoc. 2016;18:e211.

12. Moreno-Santos I, Pérez-Belmonte LM, Cabrera-Bueno F, Sánchez-Espin G, Castellano D, Such M, et al. Echocardiographic epicardial adipose tissue is related to anthropometric volume and coronary vasomotor function. Am J Cardiol. 2014;113:90–7.

13. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011;473:317–28.

14. El-Maghrabi MR, Amri EZ, Lezine G, Grimaldi PA. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. J Biol Chem. 1993;268:17665–8.

15. Amini ER, Buyukkaya E, Yilmaz E, Yildirim F, Erman S, et al. Detection of circulating oxidized LDL is a useful marker for identifying patients with coronary artery disease. Arterioscler Thromb Vasc Biol. 2001;21:844–8.

16. Keaney JF Jr, Larson MG, Vasan RS, Wilson PW, Lipska I, Corey D, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. Arterioscler Thromb Vasc Biol. 2003;23:434–9.

17. Santos-Gallego GG, Picatoste B, Badmén JJ. Pathophysiology of acute coronary syndrome. Curr Atheroscler Rep. 2014;16:401.

18. Atrobusi P, Civeira F, Recalde D, García-Oliva AL, Jaraeta E, Marzo J, et al. Individual variation of scavenger receptor expression in human macrophages with oxidized low-density lipoprotein is associated with a differential inflammatory response. J Immunol. 2007;179:3242–8.

19. Abumrad NA, El-Waghrah MR, Amri EZ, Lezine G, Grimaldi PA. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. J Biol Chem. 1993;268:17665–8.

20. Zani IA, Stephen SL, Mughal NA, Russell D, Homer-Vanniasinkam S, Wheatcroft SB, et al. Scavenger receptor structure and function in health and disease. Cell. 2015;164:109–70.
epicardial adipose tissue and coronary atherosclerosis based on computed tomography analysis. Atherosclerosis. 2015;243:293–9.

45. Antonopoulos AS, Sanna F, Sabharwal N, Thomas S, Dikonomou EK, Herdman L, et al. Detecting human coronary inflammation by imaging perivascular fat. Sci Transl Med. 2017;9:398.

46. Hansen SW, Ohtani K, Roy N, Wakamiya N. The collectins CL-L1, CL-K1 and CL-P1, and their roles in complement and innate immunity. Immunobiology. 2016;221:1058–67.

47. Roy N, Ohtani K, Hidaka Y, Amano Y, Matsuda Y, Mori K, et al. Three pentraxins C-reactive protein, serum amyloid p component and pentraxin 3 mediate complement activation using Collectin CL-P1. Biochim Biophys Acta. 2017;1861:1–14.

48. Jang S, Ohtani K, Fukuoh A, Yoshizaki T, Fukuda M, Motomura W, et al. Scavenger receptor collectin placenta 1 (CL-P1) predominantly mediates zymosan phagocytosis by human vascular endothelial cells. J Biol Chem. 2009;286:3956–65.

49. Canton J, Neculai D, Grinstein S. Scavenger receptors in homeostasis and immunity. Nat Rev Immunol. 2013;13:621–34.

50. ESC Guidelines on diabetes. Prediabetes and cardiovascular diseases. Eur Heart J. 2013;34:3035–87.

51. Castelvecchio S, Menicanti L, Garatti A, Tramarin R, Völpe M, Parolari A, et al. Myocardial revascularization for patients with diabetes: coronary artery bypass grafting or percutaneous coronary intervention? Ann Thorac Surg. 2016;102:1012–22.

52. Tu B, Rich B, Labos C, Brophy JM. Coronary revascularization in diabetic patients: a systematic review and Bayesian network meta-analysis. Ann Intern Med. 2014;161:724–32.

53. Jiménez-Navarro MF, López-Jiménez F, Bariness G, Lennon RJ, Sandhu GS, Prasad A. Long-term prognosis of complete percutaneous coronary revascularization in patients with diabetes and multivessel disease. Heart. 2015;101:1233–9.