

ORIGINAL ARTICLE

Epicardial, pericardial fat and glucagon-like peptide-1 and -2 receptors expression in stable patients with multivessel coronary artery disease: an association with the renin-angiotensin-aldosterone system

Maciej Haberka¹, Aleksander Siniarski^{2,3}, Grzegorz Gajos^{2,3}, Grzegorz Machnik⁴, Adam Kowalówka⁵, Marek Deja⁵, Bogusław Okopień⁴, Zbigniew Gąsior¹

1 Department of Cardiology, School of Health Sciences, Medical University of Silesia, Katowice, Poland

2 Department of Coronary Disease and Heart Failure, Institute of Cardiology, Jagiellonian University Medical College, Kraków, Poland

3 John Paul II Hospital, Kraków, Poland

4 Department of Internal Medicine and Clinical Pharmacology, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

5 Department of Cardiac Surgery, School of Medicine in Katowice, Medical University of Silesia, Katowice, Poland

KEY WORDS

epicardial fat, glucagon-like peptide receptors, pericardial fat, renin angiotensin aldosterone

EDITORIAL

by Iacobellis, see p. 224

Correspondence to:

Aleksander Siniarski, MD, PhD,
Department of Coronary Disease
and Heart Failure, Institute of
Cardiology, Jagiellonian University
Medical College, ul. Prądnicka 80,
31-202 Kraków, Poland, phone:
+48 12 614 22 18, email:
aleksandersiniarski@gmail.com

Received: October 10, 2020.
Revision accepted:
January 31, 2021.

Published online: February 5, 2021.
Pol Arch Intern Med. 2021;
131 (3): 233-240
doi:10.20452/pamw.15797
Copyright by the Author(s), 2021

ABSTRACT

INTRODUCTION The expression of glucagon-like peptide receptors (GLP-Rs) in epicardial fat (EF) and pericardial fat (PF) depots might be involved in the pathogenesis of cardiovascular diseases.

OBJECTIVES We sought to evaluate the messenger RNA (mRNA) expressions of GLP-1R and GLP-2R in EF and PF and their associations with the renin-angiotensin-aldosterone system (RAAS) in patients with multivessel coronary artery disease (CAD).

PATIENTS AND METHODS Consecutive stable patients with multivessel CAD requiring elective coronary artery bypass grafting were enrolled. Clinical data, anthropometric parameters, and the quantity of fat depots (assessed by cardiovascular magnetic resonance and abdominal ultrasound) were obtained. Fat samples (EF, PF, subcutaneous fat) were taken from patients during cardiac surgery. Relative mRNA expression of GLP-1R, GLP-2R, and RAAS components (angiotensin II receptor type 1, angiotensinogen, angiotensin I-converting enzyme 1, and angiotensin I-converting enzyme 2) were assessed in those fat depots.

RESULTS Fifty-three patients (64.7 [7.4] years) were included in the final analysis. We found that only the relative expression of GLP-2R was lower in PF compared with subcutaneous fat (reference). Ultrasound abdominal fat depots were associated with both GLP-1R and GLP-2R in PF. GLP-1R and GLP-2R showed significant correlations with RAAS components in both EF and PF.

CONCLUSIONS In stable patients with multivessel CAD, the relative mRNA expression for both GLP receptors was significantly associated with the majority of the analyzed RAAS components.

INTRODUCTION Epicardial fat (EF) is a visceral adipose tissue depot surrounding coronary arteries that has the unique embryology, anatomy, and functionality.¹ The increased volume of EF was found to be a risk factor for cardiovascular diseases.^{2,3} As we described previously, among patients with advanced coronary artery disease (CAD), type 2 diabetes (T2D) was associated with

an increased volume of EF and a dysfunctional profile of gene expression in this fat depot.⁴ Moreover, major differences were found in the gene expression of inflammatory markers in both EF and pericardial fat (PF) depots.⁴ The glucagon-like peptide-1 (GLP-1) is an incretin hormone with a spectrum of pharmacological and metabolic effects: an increase in glucose-dependent

WHAT'S NEW?

The expression of glucagon-like receptors (GLP-R) in epicardial fat (EF) and pericardial fat (PF) depots might be involved in the pathogenesis of cardiovascular diseases. The aim of the present study was to evaluate the relative expression of GLP-1R and GLP-2R messenger RNA (mRNA) in EF and PF and their associations with the renin-angiotensin-aldosterone system (RAAS). First, the expression of GLP receptor mRNA was present in EF and PF. To our knowledge, this is the first study to demonstrate that the expression of GLP-1R and GLP-2R mRNA was associated with RAAS components. Moreover, in patients with coronary artery disease, RAAS components were expressed in EF. Additionally, the expression of GLP-2R was lower in PF as compared with subcutaneous fat. Finally, we found that ultrasound visceral fat ratios were also associated with GLP-1 and GLP-2 receptors.

insulin secretion and in natriuresis and diuresis, a decrease in glucagon production and gastric emptying, and therefore, inhibition of food intake.⁵⁻⁷ Recently, a large clinical trial showed a significant reduction of cardiovascular mortality in patients with T2D and high cardiovascular risk by one of glucagon-like peptide-1 analogues, which could be partly associated with targeting GLP-1R.^{8,9} The expressions of GLP-1R or GLP-2R in fat depots and their relation to the renin-angiotensin-aldosterone system (RAAS) are unknown. Therefore, we sought to determine the mRNA expression of GLP-1R and GLP-2R in EF and PF depots obtained from patients with multivessel CAD. Furthermore, we hypothesized that mRNA expression of both GLP-1R and -2R would be associated with RAAS components.

PATIENTS AND METHODS **Study population** Study patients were screened and recruited at the Department of Cardiology and the Department of Cardiac Surgery of the Medical University of Silesia in Katowice. A total of 56 consecutive patients with chronic coronary syndromes scheduled for elective coronary artery bypass grafting (CABG) were included in the study and finally 53 were included in the study group. Three patients did not complete the protocol (change in the treatment schedule) and were excluded from further analysis. The study protocol and methods were described in details in our previous paper.⁴ Briefly, the study included clinical assessment, anthropometric parameters, quantification of various fat depots in ultrasound and cardiovascular magnetic resonance (CMR), and fat samples obtained during cardiac surgery. All patients had standardized pharmacotherapy according to the current European Society of Cardiology guidelines.¹⁰⁻¹³

The main exclusion criteria were defined as follows: acute coronary syndrome, congestive heart failure, left or right ventricular systolic dysfunction, indications for any other type of cardiac surgery, infectious or chronic inflammatory diseases in prior 2 months, neoplastic diseases (diagnosis and/or treatment in prior 5 years), significant renal or liver dysfunction, any anti-inflammatory or

anti-infective medicines in prior month, secondary causes of obesity, specific interventions aimed at obesity, unintentional weight loss or malnutrition, and contraindications to CMR.

The study was approved by the local Medical University of Silesia Ethics Committee (KNW/0022/KB1/127/I/13/14) and all individuals gave their written informed consent to the protocol.

Clinical characteristics Cardiovascular diseases and risk factors were defined according to the European Society of Cardiology guidelines¹⁴ and identified based on prior diagnosis and/or current treatment. Type 2 diabetes was determined based on fasting plasma glucose levels of 126 mg/dl or greater, and/or glycated hemoglobin of 6.5% or greater, or current antidiabetic treatment.¹⁵ All medical records and medications used were determined by a physician using a structured medical questionnaire.

Ultrasound measurements Ultrasound (GE Vivid 9, Milwaukee, Wisconsin, United States) was used in all patients to assess the abdominal subcutaneous (7.5 MHz transducer) or visceral (3.5 MHz transducer) fat depots. As described before, the following ultrasound measures were obtained: abdominal subcutaneous fat (ASF), abdominal preperitoneal fat thickness (APFT), and visceral intra-abdominal thickness (IAT).^{16,17} The following ratios were used to assess the relative amounts of visceral adipose tissue depots and cardiovascular risk. To demonstrate the association between IAT to ASF and APFT to ASF, we calculated the IAT to ASF and APFT to ASF ratios.

Cardiac magnetic resonance imaging of epicardial and pericardial fat Prior to sample collection, the EF and PF volumes were quantified on electrocardiography-gated cine images acquired on a 1.5 T system (GE Optima MR450w, GE Healthcare, Waukesha, Wisconsin, United States) with a dedicated cardiac coil using a steady-state free precession sequence. EF and PF were identified as the adipose tissue depot in end diastole (EF, between myocardium and visceral layer of pericardium; PF, all the fat surrounding the heart outside EF) along the walls of the right and left ventricles starting from the basal segments (atrioventricular valves level) up to the apex area. Particular fat volumes were traced manually and calculated using the summation of disc method with the scan parameters as described previously.⁴

Fat tissue collection The fat tissue samples were taken at the beginning of the CABG procedure from tissues that had not been traumatized. All the samples were taken in the same order and locations in all patients: 1) SF at sternotomy, 2) PF within the thorax, and 3) EF adjacent to the proximal right coronary artery. The biopsy specimens were immediately stored at -80 °C until further analysis.

RNA extraction and reverse transcription–polymerase chain reaction

RNA was extracted using the phenol-chloroform method using the TRI Reagent (MRC Inc., Cincinnati, Ohio, United States).¹⁸ Fat tissue samples (200 mg each) were put into a 2-ml Eppendorf-type tube directly into 1 ml of the TRI reagent, which contained 5 μ l of the precipitation carrier reagent (MRC Inc.). Thereafter, samples were homogenized by means of a rotor/stator, hand-held homogenizer (IKA-Werke GmbH & Co KG, Staufen, Germany). All subsequent steps were performed according to the manufacturer's instruction. Finally, purified RNA was resolved in 100 μ l of nuclease-free water. The RNA concentration was determined spectrophotometrically by measuring the absorbance at 260 nm (BioPhotometer, Eppendorf, Hamburg, Germany). A 500 ng of total RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Warsaw, Poland) in a reaction volume of 20 μ l. Finally, the reverse transcription reaction mixture was diluted 1:4 with nuclease-free water. A quantitative analysis of the above genes was carried out by a 2-step real-time reverse transcription–quantitative polymerase chain reaction (real-time RT-qPCR) assay. The human glyceraldehyde 3-phosphate dehydrogenase gene (*GAPDH*) was used as a reference for quantitative analysis. An amount of 2 μ l of the reverse transcription reaction mixture (ie, an equivalent of 10 ng of total RNA) was used as a template for RT-qPCR. Real-time RT-qPCR was performed using the SYBR Select Master Mix (Thermo Fisher Scientific) in a total volume of 20 μ l that contained 200 nM of each (forward and reverse) gene-specific primers. All primers for RT-qPCR were derived from the PrimerBank database (<https://pga.mgh.harvard.edu/primerbank/>).¹⁹ All primer pairs spanned the intron/exon boundary so this excluded the possibility of false-positive results from genomic DNA. All primers used are demonstrated in Supplementary material. Reactions were performed using the Roche LightCycler 480 Instrument II (Roche Diagnostics, Warsaw, Poland) with a thermal profile set as follows: 94 °C/3 min then 35 cycles of 94 °C/30 sec, 58 °C/30 sec, 72 °C/45 sec. The specificity of each reaction was confirmed by a melting-curve analysis. The reaction conditions for real-time quantitative PCR were the same for all analyzed genes. The increase in fluorescence was measured in real time and cycle threshold (CT) values were obtained. The target gene CT number was normalized to the endogenous reference *GAPDH* and the formula $2^{-(\Delta\Delta Ct)}$ was used to calculate the relative (to subcutaneous fat [SF]) gene expression.²⁰

The above-mentioned methods were used to determine relative mRNA expression of GLP-1R and GLP-2R as well as RAAS components, that is, angiotensin II receptor type 1 (AGTR1), angiotensinogen (AGT), angiotensin-I-converting enzyme 1 (ACE1), and angiotensin-I-converting enzyme 2 (ACE2).

Statistical analysis The results are expressed as means (SD) for normally distributed variables, medians (interquartile ranges [IQRs]) for non-normal distribution, or numbers (percentages). The distribution was tested for the normality with the Kolmogorov–Smirnov test. Baseline clinical parameters and the measures were compared between the subgroups using the paired *t* tests for normally distributed continuous variables; in case of non-normal distribution, the Wilcoxon rank sum test was used. Associations between parameters (genes expressions obtained from the formula $2^{-(\Delta\Delta Ct)}$) were assessed using the Pearson or Spearman rank correlation analysis depending on normal and non-normal data distribution, and are shown as *r* and *R* correlation coefficients, respectively. The relative mRNA expressions were described as medians and 95% CIs. A *P* value of less than 0.05 was considered statistically significant. Statistical analysis was performed using the Statistica software (version 10.0, Stat Soft, Kraków, Poland).

RESULTS Study group characteristics The final analysis included 53 patients (mean [SD] age, 64.7 [7.4] years; 75% men) with multivessel CAD requiring CABG and multiple cardiovascular risk factors (mean [SD], 5.5 [1.4]). In brief, all patients had hypertension and dyslipidemia. The majority of the study group were overweight (53%) or obese (39%), and almost half of them (49%) had T2D (TABLE 1). All patients had preserved left ventricular ejection fraction (mean [SD], 55.3% [4%]) and no other primary cardiomyopathy, which was evidenced in CMR. All patients received guideline-based pharmacotherapy including acetylsalicylic acid, angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker, β -blocker, and statin (TABLE 1). Treatment of T2D was also specified in TABLE 1.

Glucagon-like peptide receptors and fat depots

GLP-1R relative mRNA expression was comparable in PF (median, 0.747; 95% CI, 0.268–1.348) and EF (median, 0.364; 95% CI, 0.088–0.947) when compared with SF (FIGURE 1A). Contrary, GLP-2R expression was decreased in PF (median, 0.167; 95% CI, 0.098–0.329) but not in EF (median, 0.412; 95% CI, 0.28–1.018) when compared with SF expression (FIGURE 1B). Relative mRNA expression of GLP-1R and GLP-2R between both fat depots (PF and EF) were similar (*P* = 0.17 and *P* = 0.07, respectively). We observed significant associations between both GLP receptors in PF (*R* = 0.6; *P* < 0.0001) and EF (*R* = 0.4; *P* < 0.01). However, there were no differences in mRNA expression of both GLP receptors when patients with T2D were compared with those without T2D and patient with BMI of 30 or greater with those with BMI less than 30 kg/m² (data not shown).

Glucagon-like peptide receptors and fat depot volume on cardiovascular magnetic resonance There were no associations between the expression of

TABLE 1 Clinical characteristics of the study group (n = 53)

Parameter	Value
Age, y, mean (SD)	64.7 (7.4)
Male sex	40 (75)
Type 2 diabetes	26 (49)
Dyslipidemia	53 (100)
Total cholesterol, mg/dl, mean (SD)	152 (34)
LDL cholesterol, mg/dl, mean (SD)	86 (29)
HDL cholesterol, mg/dl, mean (SD)	43 (11)
Triglycerides, mg/dl, mean (SD)	119 (50)
Hypertension	53 (100)
Smoker or ex-smoker ^a	27 (51)
Risk factors ^b , mean (SD)	5.5 (1.4)
Number of vessels with CAD ^c , mean (SD)	2.7 (0.5)
Prior MI	9 (17)
Body mass index, kg/m ² , mean (SD)	29.9 (4.5)
Overweight/obesity	28 (53)/21 (39)
Body fat, %, mean (SD)	32.2 (7.7)
WC >80 cm in W or >94 cm in M	42 (79)
WC, cm, mean (SD)	102.4 (12.6)
Metabolic syndrome	30 (56)
Cardiovascular magnetic resonance	
Epicardial fat volume, ml, median (IQR)	90.4 (68.1–111.2)
Pericardial fat volume, ml, median (IQR)	121.7 (79.8–168)
Ultrasound	
Abdominal subcutaneous fat, mm, mean (SD)	22.7 (9.8)
Abdominal visceral fat, mm, mean (SD)	49 (27)
Abdominal preperitoneal fat, mm, mean (SD)	20.5 (6.4)
Cardiovascular pharmacotherapy	
Acetylsalicylic acid	53 (100)
β-Blocker	53 (100)
ACEI or ARB	53 (100)
Statin	53 (100)
CCB	21 (39)
Diuretics	11 (21)
Insulin	6 (9)
Oral antidiabetic medications	21 (40)
Metformin	11 (20)
Glucagon-like peptide-1 analogues	0
SGLT-2 inhibitor	1 (2)
Acarbose	3 (6)
Sulphonylurea	6 (12)

Data are presented as median (interquartile range) or number (percentage) unless otherwise indicated.

- a** Current smoking or smoking in the past for at least 1 year
- b** Risk factors: male sex, age >55 years old, hypertension, hyperlipidemia, obesity, type 2 diabetes, chronic kidney disease, smoking
- c** Number of coronary arteries with ≥50% stenosis

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CAD, coronary artery disease; CCB, calcium channel blocker; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M, men; MI, myocardial infarction; SGLT-2, sodium-glucose cotransporter-2; W, women; WC, waist circumference

both GLP receptors and the quantity of the corresponding fat depots: EF or PF volumes assessed in CMR (all $P > 0.8$). Furthermore, the IAT to ASF ratio was not associated with the mRNA expression of GLP-1R ($R = 0.1$; $P = 0.55$) and GLP-2R ($R = 0.1$; $P = 0.4$) in PF. Additionally, the APFT to ASF ratio was also not associated with GLP-1R ($R = 0.2$; $P = 0.2$) and GLP-2R ($r = 0.3$; $P = 0.07$) expression in PF.

Renin-angiotensin-aldosterone system receptors

The relative mRNA expression of ACE1 in EF (median [IQR], 0.771 [0.31–1.65]) was lower compared with SF ($P = 0.002$) and similar compared with PF (median [IQR], 0.865 [0.38–2.45]; $P = 0.5$). However, the mRNA expression of ACE2 showed no differences (all $P > 0.2$) in EF (median [IQR], 0.818 [0.13–2.63]), PF (median [IQR], 0.862 [0.15–1.76]) or SF (1.0). The AGT mRNA expressions were similar in both EF and PF (median [IQR], 1.82 [0.34–6.41] vs 1.73 [0.69–4.23]; $P = 0.9$) and significantly higher compared with SF ($P = 0.04$).

Finally, the mRNA expression of AGTR1 was lower in EF compared with PF (median [IQR], 0.58 [0.32–1.47] vs 1.24 [0.72–1.87]; $P = 0.01$) and both showed significant differences compared with SF (both $P = 0.02$). There were also no differences in mRNA expression of RAAS receptors in patients with T2D when compared with those without T2D and in patients with BMI of 30 kg/m² or greater when compared with those with BMI less than 30 kg/m² (data not shown).

Glucagon-like peptide receptors and renin-angiotensin-aldosterone system receptors

Both relative mRNA expression of GLP-1R and GLP-2R revealed significant associations with RAAS components. GLP-1R was significantly correlated with all assessed RAAS components in EF (AGTR1, AGT, ACE1, ACE2) and with: AGTR1, ACE1, and ACE2 in PF. In turn, GLP-2R was associated with ACE1 and ACE2 in PF, and with all assessed RAAS component in EF (TABLE 2). The strongest correlations for GLP-1R were observed with ACE1 in EF and PF; and for AGT in PF (all $R = 0.5$). The expression of AGTR1 in EF showed the strongest association with GLP-2R expression. In brief, the associations between relative mRNA expression of GLP receptors and RAAS components were observed for EF as well as for PF depot (TABLE 2).

DISCUSSION Our study evaluated the clinical significance of GLP-1R and GLP-2R relative mRNA expression in EF and PF in relation to the quantity of various thoracic and abdominal fat depots and expression of RAAS components in a group of patients with a very high cardiovascular risk. It was shown that GLP-1 agonist could have many potential beneficial effects on the vascular endothelium, directly improving cardiovascular outcomes in patients with T2D, for example, an antiproliferative effect on vascular smooth cells and endothelial cells, a reduction of oxidative stress,

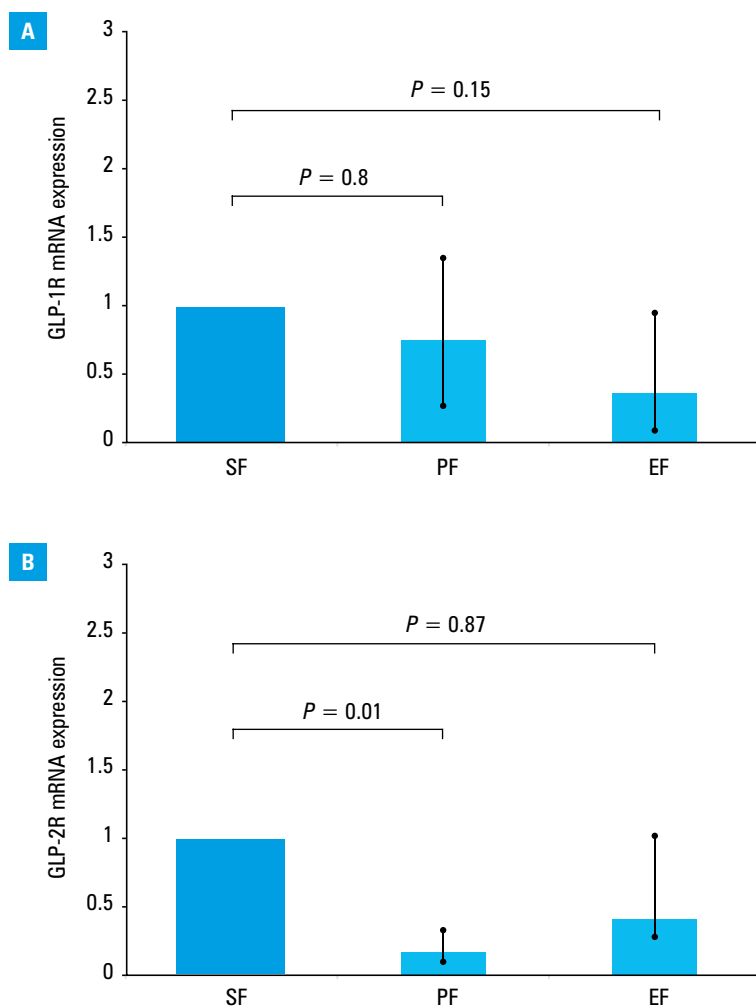


FIGURE 1 The messenger RNA (mRNA) expression of glucagon-like peptide-1 receptor (GLP-1R) in subcutaneous, pericardial, and epicardial fat depots: **A** – relative mRNA expression of GLP-1R; **B** – relative mRNA expression of glucagon-like peptide-2 receptor (GLP-2R). The values are expressed as relative mRNA expression (subcutaneous fat [SF] value used as the reference). The bars represent median values and 95% CI. Difference of mRNA expression was compared by the Wilcoxon rank sum test. Abbreviations: EF, epicardial fat; PF, pericardial fat

and an increase in nitric oxide production, resulting in a potential antiatherogenic effect.^{21,22}

Epicardial fat It was demonstrated that atherosclerotic plaques in coronary arteries were more prevalent in segments surrounded by EF.^{23,24} Epicardial fat is located directly nearby the myocardium and coronary arteries, so the beneficial effects of GLP-1 activation may extend to the heart.⁸ It has been hypothesized that the proximity of EF to the coronary arteries could promote the development of atherosclerosis via several para- and endocrine effects.^{23,24} We have shown previously that patients with CAD and T2D had an increased volume and more dysfunctional profile of gene expression in EF.⁴ Moreover, those patients had a significantly decreased expression of cardioprotective fibroblast growth factor 21.⁴ Therefore, our findings suggest the distinctive properties and importance of EF in the progression of CAD in patients with concomitant T2D.²⁴

Glucagon-like peptides receptors and epicardial fat

Iacobellis et al¹ were the first to demonstrate the presence of GLP-1R and GLP-2R expression in EF. Interestingly, GLP-2R expression was found to be 5-fold higher than GLP-1R.¹ However, in the immunofluorescence assay, the authors showed that GLP-1R was more abundant in EF than SF.¹ Contrary, in our study we found that only the relative mRNA expression of GLP-2R was lower in SF when compared with SF. Iacobellis et al¹ found no differences in GLP-1R and GLP-2R relative expression in EF depots between patients with and without T2D, which is similar to our results. Interestingly, among various medications (statins, insulin, thiazolidinediones, dipeptidyl peptidase-4 inhibitors), GLP-1 analogues were evidenced to provide a major reduction in EF volume.^{1,25-27} Dozio et al⁸ showed that GLP-1R expression in EF was associated with genes promoting beta-oxidation, white-to-brown adipocyte differentiation, and inversely associated with genes promoting pro-adipogenesis. On the other hand, GLP-2R inversely correlated with β -oxidation, and was associated with adipogenesis and lipid synthesis genes expression.⁸ Importantly, GLP-1R and GLP-2R expressions in EF were higher in patients with CAD than healthy controls and in patients with greater EF thickness.⁸ We used a reference method of EF quantification (CMR) and we found no associations between the expression of GLP receptors in fat depot and the volume of the depot. GLP-1R expression in EF was found to be lower than GLP-2R.⁸ This could be due to the down-regulation of robust cellular activities or gene enrichment suppression in subjects with chronic CAD.²⁸ Finally, we found similar expression of both analyzed receptors.

The renin-angiotensin-aldosterone system and epicardial fat

The RAAS is a family of peptides with proven endocrine and paracrine functions.²⁹ Angiotensinogen is converted to angiotensin I and angiotensin II respectively by 2 enzymes: renin and angiotensin-converting enzyme (ACE).²⁹ Importantly, angiotensin II is an active peptide which is involved in the regulation of blood pressure and fluid balance.²⁹ It was demonstrated that increased EF inflammation in an animal model of obese mice (lacking ACE2) resulted in worsening of insulin resistance and therefore impaired cardiac metabolism. Those mechanisms were associated with increased lipotoxicity and oxidative stress.^{29,30} Moreover, in a rat model after myocardial infarction, the administration of angiotensin (1-7) reduced EF inflammation, lipotoxicity, and oxidative stress resulting in improved cardiac metabolism and cardiac muscle function.³¹ It was also shown in human EF that the upregulation of ACE2 was associated with obesity and cardiac dysfunction.³⁰

Glucagon-like peptides and the renin-angiotensin-aldosterone system

The associations between glucagon-like peptides and the RAAS are mainly

TABLE 2 Glucagon-like peptide receptors and the renin-angiotensin-aldosterone system

RAAS components	Glucagon-like peptide-1 receptor		Glucagon-like peptide-2 receptor	
	Spearman rank correlation coefficient (<i>R</i>)	<i>P</i> value	Spearman rank correlation coefficient (<i>R</i>)	<i>P</i> value
Pericardial fat				
Angiotensin II receptor type 1	0.45	0.001	0.3	0.06
Angiotensinogen	0.5	<0.001	0.35	0.2
Angiotensin I-converting enzyme 1	0.5	<0.0001	0.4	<0.01
Angiotensin I-converting enzyme 2	0.4	<0.001	0.5	0.001
Epicardial fat				
Angiotensin II receptor type 1	0.35	0.01	0.6	<0.001
Angiotensinogen	0.2	0.2	0.35	0.01
Angiotensin I-converting enzyme 1	0.5	<0.01	0.4	0.02
Angiotensin I-converting enzyme 2	0.4	<0.01	0.45	0.001

Abbreviation: RAAS, renin-angiotensin-aldosterone system

based on studies in animal models. In a rodent model of polycystic ovary syndrome (PCOS) in which cardiometabolic complications are also observed, the authors analyzed the association of GLP-1 agonist treatment on mRNA expression of RAAS components in the kidney tissue.³² The GLP-1 agonist decreased renal cortical and medullary mRNA expression of RAAS components (renin and AGTR1), but only in the control group (initially, RAAS components were higher in controls compared with PCOS).³² Additionally, a GLP-1 agonist lowered renal ACE mRNA expression in the cortex and medulla of the control group but had no effect in the PCOS group.³²

In humans, it was demonstrated that GLP-1 blocked the angiotensin II-induced mesangial cell injury and had a beneficial effect in patients with diabetic nephropathy.³³ Skov et al³⁴ analyzed the impact of GLP-1 infusion using a randomized, double-blind trial on kidney function and RAAS components. GLP-1 administration had no significant effect on either glomerular filtration rate or renal plasma flow.³⁴ Despite a significant decrease in angiotensin II, renin, aldosterone, and the angiotensinogen excretion did not change.³⁴

Glucagon-like peptides receptors and other fat depots

A recent large randomized clinical trial (LEADER [Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results]) showed that GLP-1 analogues provided significant benefits in reducing cardiovascular mortality, but the mechanisms behind this effect remain poorly investigated.⁹ Ejarque et al³⁵ assessed the expression of GLP-1R in subcutaneous and visceral adipose tissue, incretin secretion, glucose homeostasis, and weight loss in patients with morbid obesity and T2D undergoing bariatric surgery. The authors showed that GLP-1R expression in adipose tissue was similar independently of T2D outcomes and was not linked with weight loss after bariatric surgery.³⁵ Nevertheless, the GLP-1R expression was higher in visceral fat than in SF.³⁵ Vendrell et al³⁶ found that GLP-1R expression in

visceral fat was associated with degree of insulin resistance. The authors showed that subjects with obesity and high degree of insulin resistance had an increased expression of GLP-1R in visceral fat.³⁶ The potential mechanisms of action of GLP-1 analogues are not yet understood, especially in terms of impact through fat depots such as EF or PF. We provide the first results showing a significant association between expression of both receptors in PF (but not EF) and ultrasound measures of abdominal visceral fat, which is in line with the above described findings.

The renin-angiotensin-aldosterone system and other fat depots

Angiotensinogen was found to be expressed in fat tissue and secreted by mature adipocytes.^{29,37} Similarly, other components of the RAAS, including AGTR1 and ACE2, were also expressed in both brown and white fat tissues.^{29,38} The AGTR1 and angiotensin II receptor type 2 may affect angiotensin II both by upregulation of the lipogenesis and downregulation of lipolysis.^{29,39} The local RAAS of the fat tissue was analyzed as a potential contributor to the development of systemic insulin resistance.⁴⁰⁻⁴² It was demonstrated that angiotensin II produced within fat tissues decreased the production of leptin and adiponectin, increased free oxygen radicals production, and finally induced insulin resistance.⁴² Higher values of angiotensin II hampered insulin signaling and increased local oxidative stress in the fat samples.⁴³

Other fat depots and cardiovascular risk assessment

Other fat depots and visceral obesity were also found to be linked with the atherosclerosis process. We demonstrated that in patients with high cardiovascular risk referred to elective coronary angiography, the carotid extra-media thickness, an index measure of perivascular adipose tissue, was an independent predictor of significant carotid stenosis.³ It was shown that a combination of ultrasound indices related to periarterial fat and the vascular wall were associated with more

complex CAD in high and very-high cardiovascular risk patients.⁴⁴ In obese patients, perivascular adipose tissue assessed by the carotid extra-media thickness was associated with a number of cardiovascular risk factors, and more importantly, with abdominal visceral fat.¹⁷

Potential mechanism It was demonstrated that GLP-1R and GLP-2R expression in EF were elevated in patients with CAD when compared with healthy controls.⁸ Moreover, targeting GLP-1R in EF, which is in close proximity to the cardiac muscle, could reduce local adipogenesis and improve fat utilization and induce brown fat differentiation, explaining the beneficial effects of GLP-1 activation.⁸ There is limited knowledge regarding the impact of GLP-2R expression in adipose tissue on cardiovascular pathophysiology. Previously, only a single paper established that link for EF.¹ Evidence from experimental studies showed that GLP-2R expression might be associated with the control of food intake and glucose homeostasis.⁴⁵

An increased GLP-2R expression was described in gastric chief cells from patients with severe obesity regardless of T2D presence, showing the potential association with total adipose tissue mass.⁴⁶ Additionally, we believe that the mechanism linking the role of the expression of the described gene mRNA with multivessel CAD is at least partly associated with inhibition of the inflammatory status in adipocytes through reduction of macrophage infiltration which possibly contributes to the improvement of insulin sensitivity.⁴⁷

Clinical implications A growing body of evidence suggests various pleiotropic effects of GLP analogues and supports their use in clinical practice. In this context of this novel and interesting clinical application, the PF or EF may be considered as a therapeutic target for GLP-1 activation.¹ Moreover, these drugs could additionally cause a significant reduction in fat quantity and inflammatory markers in EF, which was independent of weight loss or glucose control.⁴⁸ We did not observe a significant difference in the mRNA GLP-1R expression between patients with and without T2D, which may allow for a broader use of GLP-1 analogues targeting EF.

To the best of our knowledge, this is the first paper to demonstrate that the expression of both GLP receptors was similar in EF and PF. Moreover, it was associated with abdominal visceral fat measures, but not with EF or PF volumes. Finally, GLP-1R and GLP-2R showed significant associations with RAAS components, in both EF and PF depots.

We have a few limitations to acknowledge. Firstly, our study had a cross-sectional design and we cannot draw conclusions on causality and prospective changes in fat depots. Secondly, there is also no control group as it is not possible to have true healthy controls with other

clinical indications for cardiac surgery. Thirdly, the volume of specimens, especially in lean non-diabetic individuals, hindered the assessment of genes at the protein levels or histological analysis. Finally, the small number of the analyzed group limits the interpretation of comparisons between patients with or without obesity and with or without T2D.

In conclusion, we demonstrated that GLP-1R and GLP-2R mRNA expression were associated with RAAS components assessed in EF and PF from patients with severe CAD. Our findings suggest the potential role of GLP receptors and RAAS components expressed in various fat depots in mediating effects of GLP analogues or RAAS inhibitors.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

ACKNOWLEDGMENTS The study was supported by a noncommercial research grant from the Medical University of Silesia (KNW-1-085/N/5/0, KNW-1-016/K/8/K; to MH) and the Polish Cardiac Society (Servier 2016). MH is the guarantor of this work and, as such, had full access to all data in the study and takes responsibility for the integrity of data and the accuracy of the data analysis.

CONTRIBUTION STATEMENT MH conceived the concept of the study, contributed to all parts of the study, drafted the manuscript; GM and BO performed all the serum and genetic laboratory tests; AK and MD obtained the fat samples; AS and GG analyzed data and improved the manuscript; ZG improved the final manuscript.

CONFLICT OF INTEREST None declared.

OPEN ACCESS This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License (CC BY-NC-SA 4.0), allowing third parties to copy and redistribute the material in any medium or format and to remix, transform, and build upon the material, provided the original work is properly cited, distributed under the same license, and used for noncommercial purposes only. For commercial use, please contact the journal office at pamw@mp.pl.

HOW TO CITE Haberka M, Siniarski A, Gajos G, et al. Epicardial, pericardial fat and glucagon-like peptide-1 and -2 receptors expression in stable patients with multivessel coronary artery disease: an association with the renin-angiotensin-aldosterone system. *Pol Arch Intern Med.* 2021; 131: 233-240. doi:10.20452/pamw.15797

REFERENCES

- 1 Iacobellis G, Camarena V, Sant DW, Wang G. Human epicardial fat expresses glucagon-like peptide 1 and 2 receptors genes. *Horm Metab Res.* 2017; 49: 625-630. [↗](#)
- 2 Dozio E, Malavazos AE, Vianello E, et al. Interleukin-15 and soluble interleukin-15 receptor α in coronary artery disease patients: association with epicardial fat and indices of adipose tissue distribution. *PLoS One.* 2014; 9: e90960. [↗](#)
- 3 Haberka M, Skilton M, Biedroń M, et al. Obesity, visceral adiposity and carotid atherosclerosis. *J Diabetes Complications.* 2019; 33: 302-306. [↗](#)
- 4 Haberka M, Machnik G, Kowalówka A, et al. Epicardial, paracardial and perivascular fat quantity, genes expression and serum cytokines in coronary artery disease and diabetes. *Pol Arch Intern Med.* 2019; 29: 129: 738-746. [↗](#)
- 5 Gajos G. Diabetes and cardiovascular disease: from new mechanisms to new therapies. *Pol Arch Intern Med.* 2018; 128: 178-186. [↗](#)
- 6 Müller TD, Finan B, Bloom SR, et al. Glucagon-like peptide 1 (GLP-1). *Mol Metab.* 2019; 30: 72-130. [↗](#)
- 7 Kim W, Egan JM. The role of incretins in glucose homeostasis and diabetes treatment. *Pharmacol Rev.* 2008; 60: 470-512. [↗](#)
- 8 Dozio E, Vianello E, Malavazos AE, et al. Epicardial adipose tissue GLP-1 receptor is associated with genes involved in fatty acid oxidation and white-to-brown fat differentiation: a target to modulate cardiovascular risk? *Int J Cardiol.* 2019; 292: 218-224. [↗](#)
- 9 Marso SP, Daniels GH, Frandsen KB, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* 2016; 375: 311-322. [↗](#)
- 10 Davies MJ, D'Alessio DA, Fradkin J, et al. Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes

- Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care*. 2018; 41: 2669-2701. [↗](#)
- 11 Valgimigli M, Bueno H, Byrne RA, et al. 2017 ESC focused update on dual antiplatelet therapy in coronary artery disease developed in collaboration with EACTS: The Task Force for dual antiplatelet therapy in coronary artery disease of the European Society of Cardiology (ESC) and of the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2018; 39: 213-260. [↗](#)
 - 12 Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. *Eur Heart J*. 2016; 37: 2999-3058. [↗](#)
 - 13 Piepoli MF, Hoes AW, Agewall S, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: the sixth joint task force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J*. 2016; 37: 2315-2381.
 - 14 Piepoli MF, Hoes AW, Agewall S, et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice. *Eur Heart J*. 2016; 37: 2315-2381. [↗](#)
 - 15 American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes – 2019. *Diabetes Care*. 2019; 42: 13-28. [↗](#)
 - 16 Vlachos IS, Hatzioannou A, Perelas A, Perrea DN. Sonographic assessment of regional adiposity. *Am J Roentgenol*. 2007; 189: 1545-1553. [↗](#)
 - 17 Haberka M, Gasior Z. Carotid extra-media thickness in obesity and metabolic syndrome: a novel index of perivascular adipose tissue. Extra-media thickness in obesity and metabolic syndrome. *Atherosclerosis*. 2015; 239: 169-177. [↗](#)
 - 18 Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. *Nat Protoc*. 2006; 1: 581-585. [↗](#)
 - 19 Wang X, Spandidos A, Wang H, Seed B. PrimerBank: a PCR primer database for quantitative gene expression analysis, 2012 update. *Nucleic Acids Res*. 2012; 40: 1144-1149. [↗](#)
 - 20 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods*. 2001; 25: 402-408. [↗](#)
 - 21 Almutairi M, Al Batran R, Ussher JR. Glucagon-like peptide-1 receptor action in the vasculature. *Peptides*. 2019; 111: 26-32. [↗](#)
 - 22 Helmstädter J, Frenis K, Filippou K, et al. Endothelial GLP-1 (glucagon-like peptide-1) receptor mediates cardiovascular protection by liraglutide in mice with experimental arterial hypertension. *Arterioscler Thromb Vasc Biol*. 2020; 40: 145-158. [↗](#)
 - 23 Iacobellis G, Malavazos AE, Corsi MM. Epicardial fat: from the biomolecular aspects to the clinical practice. *Int J Biochem Cell Biol*. 2011; 43: 1651-1654. [↗](#)
 - 24 Nosalski R, Alsheikh EO, Guzik TJ. Diabetes enhances epicardial fat dysfunction. *Pol Arch Intern Med*. 2019; 129: 733-734. [↗](#)
 - 25 Elisha B, Azar M, Taleb N, et al. Body composition and epicardial fat in type 2 diabetes patients following insulin detemir versus insulin glargine initiation. *Horm Metab Res*. 2015; 48: 42-47. [↗](#)
 - 26 Lima-Martínez MM, Paoli M, Rodney M, et al. Effect of sitagliptin on epicardial fat thickness in subjects with type 2 diabetes and obesity: a pilot study. *Endocrine*. 2016; 51: 448-455. [↗](#)
 - 27 Iacobellis G. Epicardial fat: a new cardiovascular therapeutic target. *Curr Opin Pharmacol*. 2016; 27: 13-18. [↗](#)
 - 28 Iacobellis G, Bianco AC. Epicardial adipose tissue: emerging physiological, pathophysiological and clinical features. *Trends Endocrinol Metab*. 2011; 22: 450-457. [↗](#)
 - 29 Patel VB, Basu R, Oudit GY. ACE2/Ang 1-7 axis: a critical regulator of epicardial adipose tissue inflammation and cardiac dysfunction in obesity. *Adipocyte*. 2016; 5: 306-311. [↗](#)
 - 30 Patel VB, Mori J, McLean BA, et al. ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. *Diabetes*. 2016; 65: 85-95. [↗](#)
 - 31 Loot AE, Roks AJM, Henning RH, et al. Angiotensin-(1-7) attenuates the development of heart failure after myocardial infarction in rats. *Circulation*. 2002; 105: 1548-1550. [↗](#)
 - 32 Torres Fernandez ED, Huffman AM, Syed M, et al. Effect of GLP-1 receptor agonists in the cardiometabolic complications in a rat model of postmenopausal PCOS. *Endocrinology*. 2019; 160: 2787-2799. [↗](#)
 - 33 Ishibashi Y, Matsui T, Ojima A, et al. Glucagon-like peptide-1 inhibits angiotensin II-induced mesangial cell damage via protein kinase A. *Microvasc Res*. 2012; 84: 395-398. [↗](#)
 - 34 Skov J, Dejgaard A, Frøkiær J, et al. Glucagon-like peptide-1 (GLP-1): effect on kidney hemodynamics and renin-angiotensin-aldosterone system in healthy men. *J Clin Endocrinol Metab*. 2013; 98: 664-671. [↗](#)
 - 35 Ejarque M, Guerrero-Pérez F, de la Morena N, et al. Role of adipose tissue GLP-1R expression in metabolic improvement after bariatric surgery in patients with type 2 diabetes. *Sci Rep*. 2019; 9: 1-9. [↗](#)
 - 36 Vendrell J, El Bekay R, Peral B, et al. Study of the potential association of adipose tissue GLP-1 receptor with obesity and insulin resistance. *Endocrinology*. 2011; 152: 4072-4079. [↗](#)
 - 37 Engeli S, Schling P, Gorzelnik K, et al. The adipose-tissue renin-angiotensin-aldosterone system: Role in the metabolic syndrome? *Int J Biochem Cell Biol*. 2003; 35: 807-825. [↗](#)
 - 38 Yvan-Charvet L, Quignard-Boulangé A. Role of adipose tissue renin-angiotensin system in metabolic and inflammatory diseases associated with obesity. *Kidney Int*. 2011; 79: 162-168. [↗](#)
 - 39 Boschmann M, Ringel J, Klaus S, Sharma AM. Metabolic and hemodynamic response of adipose tissue to angiotensin II. *Obes Res*. 2001; 9: 486-491. [↗](#)
 - 40 Cooper ME. The role of the renin-angiotensin-aldosterone system in diabetes and its vascular complications. *Am J Hypertens*. 2004; 17: 16-20. [↗](#)
 - 41 Kim S, Soltani-Bejnoud M, Quignard-Boulange A, et al. The adipose renin-angiotensin system modulates systemic markers of insulin sensitivity and activates the intrarenal renin-angiotensin system. *J Biomed Biotechnol*. 2006; 2006: 1-6. [↗](#)
 - 42 Roubíček T, Dolínková M, Bláha J, et al. Increased angiotensinogen production in epicardial adipose tissue during cardiac surgery: possible role in a postoperative insulin resistance. *Physiol Res*. 2008; 57: 1-18.
 - 43 Ferder L, Inserra F, Martínez-Maldonado M. Inflammation and the metabolic syndrome: Role of angiotensin II and oxidative stress. *Curr Hypertens Rep*. 2006; 8: 191-198. [↗](#)
 - 44 Haberka M, Lelek M, Bochenek T, et al. Novel combined index of cardiometabolic risk related to periaarterial fat improves the clinical prediction for coronary artery disease complexity. *Atherosclerosis*. 2018; 268: 76-83. [↗](#)
 - 45 Guan X, Shi X, Li X, et al. GLP-2 receptor in POMC neurons suppresses feeding behavior and gastric motility. *Am J Physiol Endocrinol Metab*. 2012; 303: 853-865. [↗](#)
 - 46 Li F, Lu L, Peng Y, et al. Increased GLP2R expression in gastric chief cells of patients with severe obesity regardless of diabetes status. *Int J Obes*. 2017; 41: 1303-1305. [↗](#)
 - 47 Lee YS, Park MS, Choung JS, et al. Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. *Diabetologia*. 2012; 55: 2456-2468. [↗](#)
 - 48 Grosso AF, De Oliveira SF, Higuchi MDL, et al. Synergistic anti-inflammatory effect: simvastatin and pioglitazone reduce inflammatory markers of plasma and epicardial adipose tissue of coronary patients with metabolic syndrome. *Diabetol Metab Syndr*. 2014; 6: 1-8. [↗](#)