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

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

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.
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 patients with coronary artery disease, RAAS components were expressed in EF. 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. 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. 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.

Clinical characteristics Cardiovascular diseases and risk factors were defined according to the European Society of Cardiology guidelines 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. 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). 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.

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). 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 μl 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 μl 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 μl. 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 μl 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 μl 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/). 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 data. 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^ΔΔCt was used to calculate the relative (to subcutaneous fat [SF]) gene expression. 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^ΔΔCt) 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
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,
Glucagon-like peptides receptors and epicardial fat

Iacobellis et al. 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. However, in the immunofluorescence assay, the authors showed that GLP-1R was more abundant in EF than SF. 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 al. 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. Dozio et al. 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. 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. 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. This could be due to the down-regulation of robust cellular activities or gene enrichment suppression in subjects with chronic CAD. 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. Angiotensinogen is converted to angiotensin I and angiotensin II respectively by 2 enzymes: renin and angiotensin-converting enzyme (ACE). Importantly, angiotensin II is an active peptide which is involved in the regulation of blood pressure and fluid balance. 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. 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. It was also shown in human EF that the upregulation of ACE2 was associated with obesity and cardiac dysfunction.

and an increase in nitric oxide production, resulting in a potential antiatherogenic effect.

**Epicardial fat**

It was demonstrated that atherosclerotic plaques in coronary arteries were more prevalent in segments surrounded by EF. Epicardial fat is located directly nearby the myocardium and coronary arteries, so the beneficial effects of GLP-1 activation may extent to the heart. It has been hypothesized that the proximity of EF to the coronary arteries could promote the development of atherosclerosis via several paracrine and endocrine effects. We have shown previously that patients with CAD and T2D had an increased volume and more dysfunctional profile of gene expression in EF. Moreover, those patients had a significantly decreased expression of cardioprotective fibroblast growth factor 21. Therefore, our findings suggest the distinctive properties and importance of EF in the progression of CAD in patients with concomitant T2D.

**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

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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. 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). Additionally, a GLP-1 agonist lowered renin ACE mRNA expression in the cortex and medulla of the control group but had no effect in the PCOS group.

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. Skov et al. 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. Despite a significant decrease in angiotensin II, renin, aldosterone, and the angiotensinogen excretion did not change.

**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. Ejarque et al. 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. Nevertheless, the GLP-1R expression was higher in visceral fat than in SF. Vendrell et al. 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. 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. Similarly, other components of the RAAS, including AGTR1 and ACE2, were also expressed in both brown and white fat tissues. The AGTR1 and angiotensin II receptor type 2 may affect angiotensin II both by upregulation of the lipogenesis and downregulation of lipolysis. The local RAAS of the fat tissue was analyzed as a potential contributor to the development of systemic insulin resistance. 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. Higher values of angiotensin II hampered insulin signaling and increased local oxidative stress in the fat samples.

**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. It was shown that a combination of ultrasound indices related to periarterial fat and the vascular wall were associated with more

| RAAS components | Glucagon-like peptide-1 receptor | Glucagon-like peptide-2 receptor |
|-----------------|---------------------------------|---------------------------------|
|                 | Spearman rank correlation coefficient ($\rho$) | $P$ value | Spearman rank correlation coefficient ($\rho$) | $P$ 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-convertase enzyme 1 | 0.5 | <0.0001 | 0.4 | <0.01 |
| Angiotensin I-convertase 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-convertase enzyme 1 | 0.5 | <0.01 | 0.4 | 0.02 |
| Angiotensin I-convertase enzyme 2 | 0.4 | <0.001 | 0.45 | 0.001 |

Abbreviation: RAAS, renin-angiotensin-aldosterone system
complex CAD in high and very-high cardiovascular risk patients.\textsuperscript{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.\textsuperscript{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.\textsuperscript{9} 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.\textsuperscript{9} 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.\textsuperscript{1} Evidence from experimental studies showed that GLP-2R expression might be associated with the control of food intake and glucose homeostasis.\textsuperscript{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.\textsuperscript{46} Additionally, we believe that the mechanism linking the role of the expression of the described gene mRNA with multivessel CAD 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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**

**ARTICLE INFORMATION**

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**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; AM 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.

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