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FULL RESEARCH PAPER
Biomarkers

Human epicardial adipose tissue expresses glucose-dependent insulinotropic polypeptide, glucagon, and glucagon-like peptide-1 receptors as potential targets of pleiotropic therapies

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Aims

Human epicardial adipose tissue (EAT) plays a crucial role in the development and progression of coronary artery disease, atrial fibrillation, and heart failure. Microscopically, EAT is composed of adipocytes, nerve tissues, inflammatory, stromovascular, and immune cells. Epicardial adipose tissue is a white adipose tissue, albeit it also has brown fat-like or beige fat-like features. No muscle fascia divides EAT and myocardium; this allows a direct interaction and crosstalk between the epicardial fat and the myocardium. Thus, it might be a therapeutic target for pharmaceutical compounds acting on G-protein–coupled receptors, such as those for glucose-dependent insulinotropic polypeptide (GIP), glucagon (GCG), and glucagon-like peptide-1 (GLP-1), whose selective stimulation with innovative drugs has demonstrated beneficial cardiovascular effects. The precise mechanism of these novel drugs and their tissue and cellular target(s) need to be better understood. We evaluate whether human EAT expresses GIP, GCG, and GLP-1 receptors and whether their presence is related to EAT transcriptome. We also investigated protein expression and cell-type localization specifically for GIP receptor (GIPR) and glucagon receptor (GCGR).

Methods and results

Epicardial adipose tissue samples were collected from 33 patients affected by cardiovascular diseases undergoing open heart surgery (90.9% males, age 67.2 ± 10.5 years mean ± SD). Microarray and immunohistochemistry analyses were performed. Microarray analysis showed that GIPR and GCGR messenger ribonucleic acids (mRNAs) are expressed in EAT, beyond confirming the previously found GLP-1 [3776 ± 1377 arbitrary unit (A.U.), 17.77 ± 14.91 A.U., and 3.41 ± 2.27 A.U., respectively]. The immunohistochemical analysis consistently indicates that GIPR and GCGR are expressed in EAT, mainly in

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macrophages, isolated, and in crown-like structures. In contrast, only some mature adipocytes of different sizes showed cytoplasmic immunostaining, similar to endothelial cells and pericytes in the capillaries and pre-capillary vascular structures. Notably, EAT GIPR is statistically associated with the low expression of genes involved in free fatty acid (FFA) oxidation and transport and those promoting FFA biosynthesis and adipogenesis ($P < 0.01$). Epicardial adipose tissue GCGR, in turn, is related to genes involved in FFA transport, mitochondrial fatty acid oxidation, and white-to-brown adipocyte differentiation, in addition to genes involved in the reduction of fatty acid biosynthesis and adipogenesis ($P < 0.01$).

Conclusions Having reported the expression of the GLP-1 receptor previously, here, we showed that GIPR and GCGR similarly present at mRNA and protein levels in human EAT, particularly in macrophages and partially adipocytes, suggesting these G-protein–coupled receptors as pharmacological targets on the ongoing innovative drugs, which seem cardiometabolically healthy well beyond their effects on glucose and body weight.

Lay summary Human epicardial adipose tissue (EAT) is a unique and multifunctional fat compartment of the heart. Microscopically, EAT is composed of adipocytes, nerve tissues, inflammatory, stromovascular, and immune cells. Epicardial adipose tissue is a white adipose tissue, albeit it also has brown fat-like or beige fat-like features. No muscle fascia divides EAT and myocardium; this allows a direct interaction and crosstalk between the epicardial fat and the myocardium. Due to its distinctive transcriptome and functional proximity to the heart, EAT can play a key role in the development and progression of coronary artery disease, atrial fibrillation, and heart failure. Clinically, EAT, given its rapid metabolism and simple measurability, can be considered a novel therapeutic target, owing to its responsiveness to drugs with pleiotropic and clear beneficial cardiovascular effects such as the glucagon-like peptide-1 receptor (GLP-1R) agonists.

- Human EAT is found to express the genes encoding the receptors of glucose-dependent insulinotropic polypeptide receptor (GIPR), glucagon receptor (GCGR), and GLP-1. The immunohistochemistry indicates that GIP and GCG receptor proteins are present in EAT samples. Epicardial adipose tissue GIPR is inversely associated with genes involved in free fatty acid (FFA) oxidation and transport and with genes promoting FFA biosynthesis and adipogenesis. Epicardial adipose tissue GCGR is correlated with genes promoting FFA transport and activation for mitochondrial beta-oxidation and white-to-brown adipocyte differentiation and with genes reducing FFA biosynthesis and adipogenesis.
- As the myocardium relies mostly on FFAs as fuel and is in direct contiguity with EAT, these findings may have a great importance for the modulation of the myocardial activity and performance. Given the emerging use and cardiovascular effects of GLP-1R agonists, dual GIPR/GLP-1R agonists, and GLP-1R/GIPR/GCGR triagonists, we believe that pharmacologically targeting and potentially modulating organ-specific fat depots through G-protein–coupled receptors may produce beneficial cardiovascular and metabolic effects.

Keywords Epicardial fat • Epicardial adipose tissue • GIP receptor • Glucagon receptor • GLP-1 receptor • Obesity • Cardiovascular diseases • Tirzepatide • Type 2 diabetes • GLP-1R agonist

Introduction

Epicardial adipose tissue (EAT) is a unique and multifunctional fat compartment of the heart.[1,2] Epicardial adipose tissue is mostly located in the atrioventricular and interventricular grooves and can be differentiated into pericoronary EAT (located directly around or on the coronary artery adventitia) and myocardial EAT (the fat depot just over the myocardium).[1] This peculiar fat depot not only is composed mainly of adipocytes but also contains nerve cells, inflammatory cells (macrophages and mast cells), stromal cells, vascular cells, and immune cells.[1] Epicardial adipose tissue not only is a white adipose tissue but also has brown fat-like and beige fat-like features.[3] No muscle fascia is present between EAT and the myocardium; therefore, the two tissues share the same microcirculation.[4] This feature is unique to EAT; no other visceral fat depot has this contiguity with the target organ. The lack of an anatomical barrier allows crosstalk between EAT and the contiguous myocardium.[1]

Due to its unique transcriptome, as compared to other fat depots, and functional proximity to the heart, EAT can play a crucial role in the onset and progression of coronary artery disease, atrial fibrillation, and heart failure.[5] Under these conditions, the physiological function of EAT of supplying the underlying myocardium with free fatty acids (FFAs) and heat is overcome leading to an ectopic fatty infiltration of the cardiomyocytes.[1,2,6] Clinically, EAT, given its rapid metabolism and simple measurability, can be considered a novel therapeutic target, owing to its responsiveness to drugs with pleiotropic and clear beneficial cardiovascular effects such as the glucagon-like peptide-1 receptor (GLP-1R) agonists.[7,8] Pharmacological modulation of G-protein–coupled receptors might regulate cardiovascular morphology and function.[9] We and others showed that either daily or weekly GLP-1R agonists induced a substantial (from 20% to 35%) reduction of EAT mass.[10–13] The presence of the GLP-1R, which we first demonstrated at messenger ribonucleic acid (mRNA) level and immunofluorescence in the human EAT, suggests that the positive cardiovascular effects of the GLP-1R agonists may be mediated at least in part through a direct action on this particular adipose depot, even if the specific cell types involved are uncharacterized.[14] We found that EAT GLP-1R activation is correlated with an up-regulation of the genes involved in FFA oxidation and brown fat differentiation.[15] Free fatty acids (FFAs) represent the primary source of energy for the myocardium at rest.[1]

More recently, a combined GLP-1R agonist and glucose-dependent insulinotropic polypeptide receptor (GIPR) agonist has been proposed as a novel therapeutic option for type 2 diabetes and obesity, with potential cardiovascular benefits.[16] Tirzepatide, a novel once-weekly dual GIP and GLP-1 receptor agonist, showed greater weight loss than semaglutide, a weekly GLP-1R agonist, interesting visceral fat reduction (SURPASS-3).[16–18] However, the mechanisms are still unclear.

Metabolic beneficial effects of GLP-1R/GIPR/glucagon receptor (GCGR) triagonists are also emerging.[19,20] Based on preclinical data, SAR441255 is a potent unimolecular triagonist that activates all three G-protein–coupled receptors *in vitro* and provides better weight loss and improved glucose control than unimolecular dual GLP-1R/GCGR agonists in mice and monkey models.[19,21] SAR441255 significantly reduced body weight via increased energy expenditure and non-fasting glucose levels in a diet-induced obese (DIO) female mice model compared with the GLP-1R/GCG dual agonism.[19] A similar experiment was reproduced in DIO and diabetic cynomolgus monkeys with no changes in body weight or in HbAc1 plasma levels between monkeys treated with SAR441255 or treated with a GLP1R/GCG dual agonist, although fasting plasma glucose was significantly lowered in SAR441255-treated animals.[19] Furthermore, SAR441255 was proved to be safe regarding glucose control in both DIO and diabetic cynomolgus monkeys and did not cause any major CV events in lean cynomolgus monkeys, which was highly relevant for further clinical development.[19]

Thus, this ongoing demonstration of the potential benefits of multiple G-protein–coupled receptor co-agonist combinations will likely lead to enhanced treatment options for patients with cardiometabolic disorders. However, whether human EAT expresses GIPR and GCGR is still unknown and unexplored.

Hence, in this study, we sought to evaluate whether human EAT expresses GIPR and GCGR, besides GLP-1R, and whether GIPR and GCGR levels are related to the expression of genes regulating FFA metabolism, adipogenesis, and white-to-brown adipocyte differentiation. We also investigated the GIPR and GCGR expression at the protein level, identifying the cell types selectively expressing the highest levels of these receptors. Pharmacologically, targeting these EAT cells may induce beneficial cardiovascular and metabolic effects.

Methods

Study population

We enrolled 33 patients who were candidate of standard of care cardiac surgery. Twenty-three patients underwent elective coronary artery bypass graft (CABG) surgery for coronary artery disease, and 10 patients underwent surgery for valve replacement (VR).

We excluded patients with the following criteria: age ≤18 years; acute myocardial infarction; history, signs, or symptoms of atrial fibrillation and/or heart failure in the previous 6 months; malignant diseases; major abdominal surgery in the last 6 months; renal and liver diseases; chronic inflammatory diseases; more than 3% change in body weight in the previous 3 months; missing or incomplete clinical history and data; and current use of GLP-1 agonists, sodium-glucose co-transporter-2 (SGLT2), and dipeptidyl peptidase-4 (DPP4) inhibitors. The local ethics committee approved the study protocol (ASL Milano Due, n. 2516), and patients gave their written informed consent to participate. The study was conducted in accordance with the Declaration of Helsinki, as revised in 2013, and Good Clinical Practice guidelines.

Biochemical parameters

Blood samples were collected after overnight fasting into pyrogen-free EDTA tubes or tubes for serum collection during hospitalization, the day before cardiac surgery. We used Cobas 6000 analyser and commercial kits (Roche Diagnostics, Milan, Italy) to quantify routine biochemical parameters, as previously reported.[15] LDL cholesterol was calculated with the Friedewald formula. The homoeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: HOMA-IR = fasting insulin [μU/mL] × fasting glucose [mmol/L] / 22.5.

Anthropometric measures

Anthropometric measures were assessed during hospitalization, the day before cardiac surgery. Body weight was measured to the nearest 0.1 kg using a balance beam scale; height was measured to the nearest 0.1 cm using a stadiometre. Waist circumference and hip circumference were measured using an unstretchable tape in a standing position without any pressure on the bodily surface, accurate to 0.1 cm. Waist circumference and hip circumference were measured using the middle of the lowest gear, of the high point of the iliac crest, and on the biggest environmental gluteal muscle, respectively. Waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) were then calculated. A waist circumference ≥102 cm for males and ≥88 cm for females indicated abdominal obesity. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) square.

Epicardial adipose tissue collection

Before starting cardiopulmonary bypass pumping, a sample of EAT adjacent to the proximal right coronary artery was harvested and stored in Allprotect Tissue Reagent (Qiagen, Hilden, Germany) at −20 °C until RNA extraction.

Epicardial adipose tissue quantification

All patients underwent standard echocardiography using commercially available equipment (Vingmed-System Five; General Electric, Horten, Norway). Epicardial adipose tissue thickness was measured according to the method first described and validated by Iacobellis *et al.*[22] Briefly, EAT was identified as the echo-free space between the myocardium's outer wall and the pericardium's visceral layer. Epicardial adipose tissue thickness was measured perpendicularly on the free wall of the right ventricle at end-systole in three cardiac cycles.

Ribonucleic acid extraction and microarray analysis

Total RNA was extracted from tissue with the RNeasy Lipid Tissue Kit according to the manufacturer's procedure (Qiagen). Ribonucleic acid concentration was quantified by NanoDrop 2000 (ThermoScientific, Wilmington, Germany), and RNA integrity was assessed using the Agilent RNA 6000 Nano kit and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Gene expression was analysed by a one-colour microarray platform (Agilent): 50 ng of total RNA was labelled with Cy3 using the Agilent Low Input Quick-Amp Labelling kit-1 colour, according to the manufacturer's directions. Complementary RNA (cRNA) was purified with the RNeasy Mini Kit (Qiagen), and the amount and labelling efficiency were measured with NanoDrop. We used an Agilent Gene Expression Hybridization Kit for hybridization and scanned it with the Agilent G2565CA Microarray Scanner System. Data were processed using Agilent Feature Extraction Software (10.7) with the single-colour gene expression protocol, and we analysed raw data with ChipInspector Software (Genomatix, Munich, Germany). In brief, raw data were normalized on the single-probe level based on the array mean intensities, and statistics were calculated using the SAM algorithm by Tusher *et al.*[23] Fold changes were calculated from normalized data.

Histology and immunohistochemistry

Samples of EAT were collected for histology and immunohistochemistry (GIPR and GCGR) as previously described.[24] Briefly, for each sample, 3 μm paraffin tissue sections were obtained, then rehydrated and reacted with 3% H_2O_2 in dH_2O for 5 min to block endogenous peroxidase, rinsed with phosphate-buffered saline (PBS), and incubated in a 2% blocking solution in PBS for 20 min. Sections were then incubated in a humid chamber overnight at 4°C with the primary antibody against GIPR (LSBio cat# LS-A3840, dilution 1:200 with antigen retrieval method by citrate buffer pH 6) and GCGR (LSBio cat# LS-A837, dilution 1:200 with antigen retrieval method by citrate buffer pH 6) in PBS. After a thorough rinse in PBS, sections were incubated in a 1:200 v/v biotinylated secondary antibody solution (Vector Laboratories, Newark, NJ, USA) in PBS for 30 min. Histochemical reactions were performed using the Vectastain ABC kit (Vector Laboratories) and ImmPACT® DAB Substrate Kit, Peroxidase (HRP) (Vector Laboratories) as the substrate. Sections were finally counterstained with haematoxylin, dehydrated, and mounted in Eukitt (Fluka, St. Louis, MO, USA). To assess the specific of the reactions, negative control was included in each set of reactions by omitting the primary antibody. In addition, a human pancreas sample for GIPR and a human liver sample for GCGR were included as positive controls in each reaction.

Statistical analysis

Quantitative variables are expressed as mean ± standard deviation (SD). Qualitative variables are summarized as numbers and percentages. Comparison between the two groups was performed by *t*-test or Mann–Whitney tests for continuous variables, and Fisher's exact test was used for nominal variables. We examined the relationship between parameters with the Spearman correlation test. Data were analysed using GraphPad Prism 9.0 biochemical statistical package (GraphPad Software, San Diego, CA, USA). A P value < 0.05 was considered significant.

Table 1 Demographic, anthropometric, clinical, and biochemical characteristics of study patients

	Total $n = 33$
Demographic information	
Age, years	67.2 ± 10.5
Sex, *n* (%)	
Male	30 (90.9)
Female	3 (9.1)
Smoking (*n*, %)	
Current smoker	15 (45.5)
Former or non-smoker	18 (54.5)
Anthropometric measures	
BMI, kg/m^2	26.8 ± 4.6
Waist circumference, cm	102.6 ± 15.4
WHtR, cm/cm	0.60 ± 0.08
WHR, cm/cm	0.98 ± 0.12
EAT thickness (mm)	6.2 ± 2.5
Biochemistry	
Fasting glucose, mg/dL	95.6 ± 29.1
Fasting insulin, µU/mL	9.2 ± 8.5
HbA1c, %	4.7 ± 1.2
HOMA index	2.1 ± 2.0
Total cholesterol, mg/dL	158.1 ± 35.1
HDL cholesterol, mg/dL	42.9 ± 11.7
Triglycerides, mg/dL	122.6 ± 49.6
C-reactive protein, mg/dL	0.95 ± 1.8
ALT/GPT, u/L	35.9 ± 33.9
AST/GOT, u/L	38.7 ± 48.5
Comorbidities, *n* (%)	
Hypertension	26 (78.8)
Dyslipidaemia	21 (63.6)
Type 2 diabetes mellitus	10 (30.3)
General obesity	3 (9.1)
Abdominal obesity	19 (57.6)
Medications, *n* (%)	
Aspirin	21 (63.6)
ACEi/ARB	25 (75.8)
Anti-diabetic agents (metformin)	8 (24.2)
β-Blockers	17 (51.5)
Calcium channel blockers	6 (18.1)
Statins	23 (69.7)

Data are expressed as *n* (%) or mean (± standard deviation). Abdominal obesity (waist circumference ≥102 cm for males, ≥ 88 cm for females); general obesity (BMI ≥ 30 kg/m^2) BMI, body mass index; WHtR, waist-to-height ratio; WHR, waist-to-hip ratio; EAT, epicardial adipose tissue; HbA1c, haemoglobin A1c; HOMA, homoeostasis model assessment of insulin resistance; ALT/GPT, alanine aminotransferase/glutamat-pyruvat-transaminase; AST/GOT, aspartate aminotransferase/glutamic-oxaloacetic transaminase; ACEi, angiotensinogen-converting enzyme inhibitor; ARB, angiotensin receptor blockade.

Results

Patient characteristics

The main demographic, anthropometric, clinical, and biochemical characteristics of the 33 enrolled patients are shown in *Table 1*.

The mean age of the overall population was 67.2 ± 10.5 years, and most patients were males (90.9%).

The prevalence of hypertension, dyslipidaemia and type 2 diabetes was 78.8% ($n = 26$ patients), 63.6% ($n = 21$ patients), and 33.3% ($n = 10$ patients), respectively. According to BMI, the prevalence of obesity was 9.1% ($n = 3$ patients), while according to waist circumference cut-off, the prevalence of abdominal obesity was 57.6% ($n = 19$ patients). The EAT thickness ranged from 3 to 10 mm (mean 6.2 ± 2.5 mm, median 7 mm).

Patients' characteristics according to surgical intervention are shown in Supplementary material online, *Table S1* of Supplementary material online, *Material*.

Glucose-dependent insulinotropic polypeptide receptor, glucagon receptor, and glucagon-like peptide-1 receptor gene expression in epicardial adipose tissue samples

Our RNA expression analysis showed that GIPR and GCGR are expressed in EAT of all patients enrolled in the study [3776 ± 1377 arbitrary unit (A.U.) and 17.77 ± 14.91 A.U., respectively]. The analysis also confirmed the expression of the GLP-1R gene (3.41 ± 2.27 A.U.), *Figure 1*.

We found that GIPR expression was 213-fold higher than GCGR ($P < 0.0001$). Glucose-dependent insulinotropic polypeptide receptor and GCGR expression were 1107 and 5.17 times higher than GLP-1R ($P < 0.0001$ for both) (*Figure 1*).

We found positive correlations between GIPR and GCGR expression (r: 0.424, $P = 0.014$) (see Supplementary material online, *Table S2*).

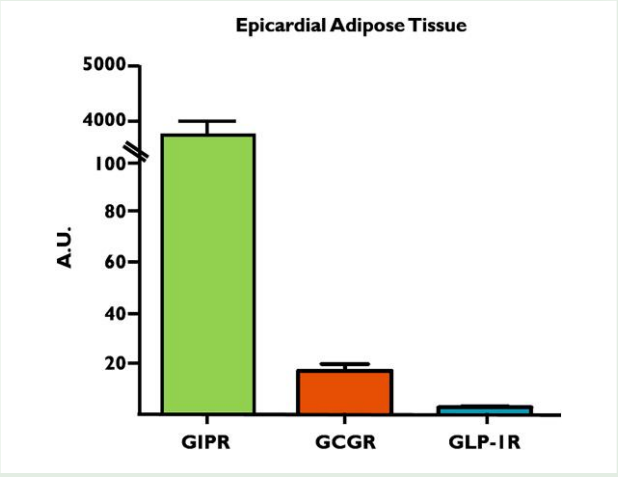

Figure 1 GIPR, GCGR, and GLP-1R expression in EAT samples in all study patients. Glucose-dependent insulinotropic peptide receptor (GIPR), glucagon receptor (GCGR), and glucagon-like peptide-1 receptor (GLP-1R) expression levels in epicardial adipose tissue (EAT) samples in all study patients; A.U., arbitrary unit.

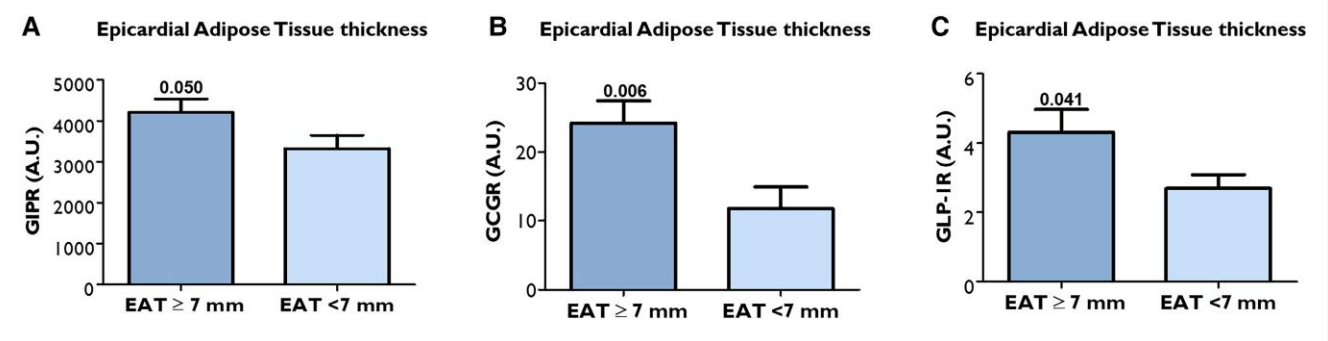

Figure 2 GIPR, GCGR, and GLP-1R gene expression in EAT samples according to ultrasound-measured EAT thickness. (*A*) Glucose-dependent insulinotropic peptide receptor (GIPR), (*B*) glucagon receptor (GCGR), and (*C*) glucagon-like peptide-1 receptor (GLP-1R) expression levels in epicardial adipose tissue (EAT) samples were compared between ultrasound-measured EAT thickness ≥or ≤ of 7 mm; A.U., arbitrary unit.

Positive correlations have also been found between GIPR and GPL-1R (*r*: 0.423, $P = 0.018$) and between GCGR and GLP-1R levels of expression (*r*: 0.354, $P = 0.005$) (see Supplementary material online, *Table S2*).

Glucose-dependent insulinotropic polypeptide receptor, GCGR, and GLP-1R expression in EAT did not differ between CABG and VR groups (GIPR: 4076 ± 1214 A.U. vs. 3087 ± 1543 A.U., fold change 1.32, $P = 0.096$; GCGR: 17.21 ± 14.64 A.U. vs. 19.06 ± 16.26, fold change: −1.018, $P = 0.860$; GLP-1R: 3.28 ± 1.87 A.U. vs. 3.72 ± 3.11, fold change: 1.055, $P = 0.799$) (see Supplementary material online, *Figure S1*).

Expression of GIPR, GCGR, and GLP-1R in EAT did not show any difference between patients with or without type 2 diabetes (GIPR: 4190 ± 1120 A.U. vs. 3597 ± 1461 A.U., fold change 1.164, $P = 0.281$; GCGR: 14.83 ± 10.74 A.U. vs. 19.62 ± 15.41 A.U., fold change −1.316, $P = 0.380$; GLP-1R: 3.17 ± 1.60 A.U. vs. 3.52 ± 2.54 A.U., fold change −1.112, $P = 0.984$) (see Supplementary material online, *Figure S2*).

Glucose-dependent insulinotropic polypeptide receptor, GCGR, and GLP-1R expression did not differ between patients with or without abdominal obesity (GIPR: 3967 ± 1169 A.U. vs. 3573 ± 1582 A.U., fold change 1.110, $P = 0.760$; GCGR: 18.57 ± 14.61 A.U. vs. 17.79 ± 14.19 A.U., fold change 1.04, $P = 0.986$; GLP-1R: 3.49 ± 2.18 A.U. vs. 3.34 ± 2.43 A.U., fold change 1.046, $P = 0.746$) (see Supplementary material online, *Figure S3*).

Glucose-dependent insulinotropic polypeptide receptor, glucagon receptor, and glucagon-like peptide-1 receptor gene expression in epicardial adipose tissue samples according to ultrasound-measured epicardial adipose tissue thickness

After classification according to the EAT median thickness (7 mm), GIPR, GCGR, and GLP-1R gene expression levels were higher in the group with the greater ultrasound-measured EAT thickness ($P = 0.050$, $P = 0.006$, and $P = 0.041$, respectively) (*Figure 2*).

Immunohistochemistry with anti-glucose-dependent insulinotropic polypeptide receptor and anti-glucagon receptor antibodies in epicardial adipose tissue samples

Of note, the immunohistochemical analysis revealed similar immunoreactivity for GIPR and GCGR proteins in EAT samples (*Figure 3*, Panels 1 and 2, respectively). Macrophages, both isolated and in crown-like structures (CLS), were always intensely positive. Only some mature adipocytes of different sizes showed immunoreactivity in their cytoplasm. Capillaries and pre-capillaries vascular structures were also positive in their wall, including endothelial cells and pericytes.

Correlation of gene expression

We investigated 84 genes involved in FFA metabolism and adipogenesis in EAT and correlated them with both GIPR and GCGR levels. All the genes included in the correlation analyses are shown in the Supplementary material online, *Material*.

Correlation of glucose-dependent insulinotropic polypeptide receptor gene expression with genes involved in fatty acid metabolism in epicardial adipose tissue samples

Fifty-five genes related to FFA metabolism were inversely correlated with EAT GIPR and 11 directly. Names, functions, and correlation parameters of FFA metabolism genes are reported in detail in *Table 2A*.

Considering the role of each gene, the observed correlations suggest both a reduced FFA transport and a reduced FFA activation for mitochondrial beta-oxidation, a reduced ketone body metabolism, and an increased regulation of FFA biosynthesis. Concerning triacylglycerol metabolism, data suggest a reduced hydrolysis of stored triglycerides and *ex novo* synthesis, along with an increased FFA release into the coronary circulation. *Figure 4A* resumes the pathophysiological significance of the observed correlations.

Correlation of glucose-dependent insulinotropic polypeptide receptor gene expression with genes involved in adipogenesis in epicardial adipose tissue samples

Fifty-eight genes related to adipogenesis were inversely correlated with EAT GIPR and five directly. Names, functions, and correlation parameters of adipogenesis genes are reported in detail in *Table 2B*.

According to these correlations, EAT GIPR is related to genes promoting adipogenesis and both reducing and promoting white-to-brown

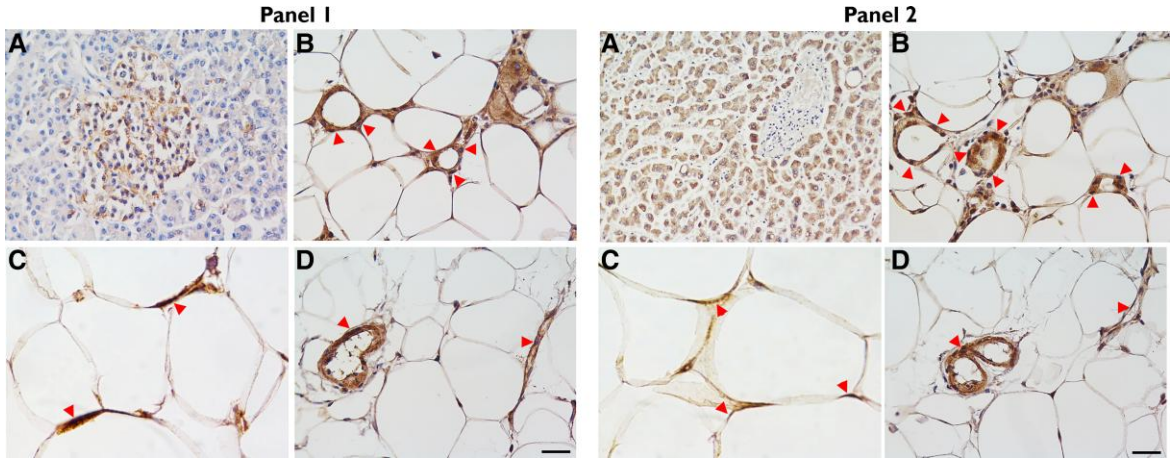

Figure 3 Immunohistochemistry with anti-GIPR and anti-GCGR antibody in EAT samples. *Panel 1* shows immunoreactivity for glucose-dependent insulinotropic peptide receptor (GIPR) proteins in epicardial adipose tissue (EAT) samples. Representative pictures (*A*) of a human pancreas sample as positive control (islets of Langerhans) and (*B*) of EAT showing immunoreactivity (arrowheads) in macrophages organized in crown-like structures (CLS), (*C*) in the cytoplasm of some adipocytes, and (*D*) in the wall of blood vessels. Scale bar: 20 μm in *A*, *B*, and *D* and 7 μm in *C*. *Panel 2* shows immunoreactivity for glucagon receptor (GCGR) proteins in EAT samples. Representative pictures (*A*) of a human liver sample as positive control (hepatocytes) and (*B*) of EAT showing immunoreactivity (arrowheads) in macrophages organized in Crown-like Structures (CLS), (*C*) in the cytoplasm of some adipocytes, and (*D*) in the wall of blood vessels. Scale bar: 20 μm in *A*, *B*, and *D* and 7 μm in *C*.

adipocyte differentiation. *Figure 4A* resumes the pathophysiological significance of the observed correlations.

Correlation of glucagon receptor gene expression with genes involved in fatty acid metabolism in epicardial adipose tissue samples

Twenty-three genes related to FFA metabolism were directly correlated with GCGR and three inversely. Names, functions, and correlation parameters of genes associated with FFA are reported in detail in *Table 2C*.

Considering the role of each gene, the observed correlations suggest an increased FFA transport, an increased FFA activation for mitochondrial beta-oxidation, an increased ketone body metabolism, and a reduced FFA biosynthesis.

Figure 4B resumes the pathophysiological significance of the observed correlations.

Correlation of glucagon receptor gene expression with genes involved in adipogenesis in epicardial adipose tissue samples

Thirty genes related to adipogenesis were directly correlated with GCGR and three inversely. Names, functions, and correlation parameters of genes related to adipogenesis are reported in detail in *Table 2D*.

According to these correlations, EAT GCGR is related to genes reducing adipogenesis and both reducing and promoting white-to-brown adipocyte differentiation. However, the major regulators of brown adipogenesis were positively correlated, such as peroxisome proliferator-activated receptor alpha (PPARA), peroxisome proliferator-activated receptor delta (PPARD), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (PPARGC1A), uncoupling protein 1 (UCP-1), PR domain containing 16 (PRDM16), and *C*-terminal SRC kinase (SRC). *Figure 4B* resumes the pathophysiological significance of the observed correlations.

Discussion

Epicardial adipose tissue dysfunction has recently emerged as a novel cardiovascular risk factor. The present study shows that human EAT expresses both GIPR and GCGR, in addition to GLP-1R, at the mRNA and protein level, confirming our previous results on the presence of the GLP-1R and extending the observation on G-protein–coupled receptors much relevant in drug development.[14,15] The accumulating demonstration of the beneficial cardiovascular effects of GLP-1R agonists, dual GIPR/GLP-1R agonists, and GLP-1R/GIPR/GCGR triagonists suggests that our findings may be of interest in explaining the unknown mechanisms of action of the novel drugs and strengthen their clinical use.

Notably, with an immunostaining analysis of EAT samples from CABG and VR patients, we identified the GIP and GCG receptors mainly in the isolated and CLS macrophages. We observed an intense GIPR and GCGR positivity in the wall, including endothelial cells and pericytes, of capillaries and pre-capillary vascular structures. In contrast, mature adipocytes of different sizes showed only a variable cytoplasmic immunoreactivity for either receptor. Crown-like structures form exclusively at sites of adipocyte death and scavenge the residual adipocyte lipid droplet.[25,26] However, CLS density is also considered a marker of adipose tissue inflammation. We have recently found a higher presence of CLS in EAT of patients with coronary artery disease than in controls.[27]

Glucagon-like peptide-1 receptor agonists have been shown in preclinical and clinical studies to inhibit multiple inflammatory pathways, so they are excellent candidates for treating cardiometabolic disorders.[1,28,29] By better understanding how these drugs work, it may be possible to identify their pleiotropic effects, which may result in protection from chronic complications, such as cardiovascular diseases. The GIP/GLP-1 and GLP-1/GIP/GCG treatments are proved to reduce markedly body weight and glucose levels.[19,21] Our previous and present

Table 2 Correlation analysis of EAT GIPR and EAT GCGR with genes involved in FFA metabolism and adipogenesis

Glucose-dependent insulinotropic peptide receptor (GIPR)

a) Fatty acid metabolism genes and glucose-dependent insulinotropic peptide receptor (GIPR)

Genes	Family group	Function	Correlation coefficient	*P* value
ACAD11	Acetyl-CoA dehydrogenases	Promote FFA oxidation	−0.7507	<0.0001
ACADS			−0.5371	0.0013
ACADVL			−0.5531	0.0008
ACADM			−0.5551	0.0008
ACAD9			−0.5899	0.0003
ACSBG1	Acyl-CoA synthetases	Activate long- and medium-chain FFA for oxidation	−0.3797	0.0293
ACSBG2			0.6129	0.0001
ACSL1			−0.4024	0.0203
ACSL3			−0.5575	0.0008
ACSL4			−0.6795	<0.0001
ACSL5			−0.5348	0.0013
ACSL6			0.5641	0.0006
ACSM4			0.5674	0.0006
ACSM5			−0.4469	0.0091
ACOT1	Acyl-CoA thioesterases	Regulate FFA oxidation in mitochondria and peroxisomes	−0.4368	0.011
ACOT12			0.3907	0.0246
ACOT6			0.4225	0.0143
ACOT7			−0.735	<0.0001
ACOT8			−0.6153	<0.0001
ACOT9			−0.7035	<0.0001
ACOX1	Acyl-CoA oxidases	The first enzyme of the fatty acid beta-oxidation pathway	−0.5194	0.002
ACOX2		Involved in the degradation of long branched fatty acids	−0.515	0.0022
ACOX3		Involved in the desaturation of 2-methyl-branched fatty acids in peroxisomes	−0.6116	0.0002
ACAT1	Acetyl-CoA transferases	Catalyses the last step of the mitochondrial beta-oxidation pathway	−0.497	0.0033
ACAA1		Involved in the beta-oxidation system of the peroxisomes	−0.6186	0.0001
ACAA2		Catalyses the last step of the mitochondrial fatty acid beta-oxidation	−0.5374	0.0013
ACAT2		Intracellular cholesterol esterification	−0.736	<0.0001
CPT1A	Fatty acid transport	Transport FFA into mitochondria for oxidation	−0.6013	0.0002
CPT1B			−0.5074	0.0026
CPT1C			−0.4131	0.0169
CPT2			−0.6003	0.0002
CRAT			−0.5916	0.0003
CROT			−0.5729	0.0005
FABP1		Facilitate FFA transport across membranes	0.538	0.0012
SLC27A1			−0.6785	<0.0001
SLC27A3			−0.7035	<0.0001
SLC27A4			−0.478	0.0003
SLC27A5			−0.4923	0.0036
FABP7		Cytoplasmatic FFA transport proteins	0.5572	0.0008
FABP2			0.5104	0.0024
FABP4			−0.5084	0.0025
FABP12			0.6093	0.0002
FABP5			−0.4686	0.006
PRKAA2	Fatty acid biosynthesis regulation	AMP-activated, alpha 2 catalytic subunit: inhibits ATP-consuming biosynthetic pathways	−0.6355	<0.0001
PRKAB1		Regulatory B1 subunit of AMP-activated protein kinase: inhibits ATP-consuming biosynthetic pathways	−0.8456	<0.0001

Continued

Table 2 *Continued*

Glucose-dependent insulinotropic peptide receptor (GIPR)

a) Fatty acid metabolism genes and glucose-dependent insulinotropic peptide receptor (GIPR)

Genes	Family group	Function	Correlation coefficient	*P* value
PRKAB2		Regulatory B2 subunit of AMP-activated protein kinase: inhibits ATP-consuming biosynthetic pathways	−0.7574	<0.0001
PRKACA		Catalytic subunits of protein kinase A: promotes fat utilization	0.6537	<0.0001
PRKACB		Catalytic subunit of cAMP (cyclic AMP)-dependent protein kinase	−0.5525	0.0009
PRKAG1		Regulatory G1 subunit of AMP-activated protein kinase: inhibits ATP-consuming biosynthetic pathways	−0.734	<0.0001
PRKAG2		Regulatory G2 subunit of AMP-activated protein kinase: inhibits ATP-consuming biosynthetic pathways	−0.4559	0.0077
ALDH2	Other fatty acid metabolism genes	Oxidizes aldehydes to generate carboxylic acids for use	−0.4078	0.0185
DECR1		Accessory enzymes that participate in the beta-oxidation and metabolism of unsaturated fatty enoyl-CoA esters	−0.5792	0.0004
ECHS1		Involved in mitochondrial fatty acid beta-oxidation pathway	−0.5137	0.0022
ECI2		Mitochondrial enzyme involved in beta-oxidation of unsaturated fatty acids	−0.6487	<0.0001
HADHA		Catalyses the last three steps of mitochondrial beta-oxidation of long chain fatty acids	−0.4682	0.006
MCEE		Involved in the degradation of odd chain-length fatty acids	−0.5745	0.0005
MUT			−0.5805	0.0004
PPA1		Catalyses the hydrolysis of pyrophosphate to inorganic phosphate	−0.5451	0.001
GK	Triacylglycerol metabolism	Esterification of FFA with reduced FFA efflux	−0.6344	<0.0001
LIPE		In adipose tissue hydrolyses stored triglycerides to free fatty acids	−0.4335	0.0117
GK2		Key enzyme in the regulation of glycerol uptake and metabolism	0.5577	0.0007
GPD1		Synthesis of triglycerides	−0.5872	0.0003
GPD2			−0.3556	0.0423
BDH2	Ketogenesis and ketone body metabolism	Involved in the synthesis and degradation of ketone bodies	−0.5254	0.0017
HMGCS1			−0.7764	<0.0001
OXCT2		Involved in ketone body catabolism.	0.3795	0.0294
HMGCL		Catalyzes the final step of leucine degradation and plays a key role in ketone body formation.	−0.6511	<0.0001

b) Adipogenis genes and glucose-dependent insulinotropic peptide receptor (GIPR)

Genes	Family group	Function	Correlation coefficient	*P* value
ADIPOQ	Adipokines and hormones	Involved in the control of fat metabolism and insulin sensitivity, with direct anti-diabetic, anti-atherogenic, and anti-inflammatory activities	−0.548	0.001
ANGPT2		An inducer of fat uptake in adipose tissue for prevention of ectopic lipid deposition	−0.538	0.001
RETN		A feedback regulator of adipogenesis	0.438	0.011
AGT		Encodes for angiotensinogen precursor angiotensin	−0.540	0.001
ADIG		Adipocyte-specific protein that plays a role in adipocyte differentiation	0.569	0.001
FASN	PPARγ TARGETS	Fatty acid synthetase that catalyses the *de novo* biosynthesis of long-chain saturated fatty acids	−0.368	0.035
LIPE		In adipose tissue hydrolyses stored triglycerides to free fatty acids	−0.434	0.012
FABP4		Lipid transport protein in adipocytes	−0.508	0.003
ADRB2	Anti-adipogenesis	Beta-2-adrenergic receptor with lipolytic activity	−0.482	0.005
CDKN1A		A cyclin-dependent kinase inhibitor	−0.510	0.002
CDKN1B			−0.678	<0.0001
DDIT3		A member of the CCAAT/enhancer-binding protein (C/EBP) family of transcription factors that block adipogenesis	−0.731	<0.0001
FOXO1		Transcription factor that inhibits lipogenesis	−0.483	0.004
HES1			−0.670	<0.0001

Continued

Table 2 *Continued*

b) Adipogenis genes and glucose-dependent insulinotropic peptide receptor (GIPR)

Genes	Family group	Function	Correlation coefficient	*P* value
LRP5		A transmembrane low-density lipoprotein receptor that binds and internalizes ligands in the process of receptor-mediated endocytosis	−0.492	0.004
NCOR2		Inhibits adipogenic differentiation by binding to PPARγ and represses the expression of PPARγ target genes	−0.743	<0.0001
SHH		Inhibits adipocyte differentiation	0.565	0.001
RUNX1T1		Recruits a range of co-repressors to acts as a negative regulator of adipogenesis	−0.636	<0.0001
SIRT1		Attenuate adipogenesis through PPARγ repressive acetylation	−0.778	<0.0001
SIRT2		Attenuate adipogenesis through PPARγ repressive acetylation	−0.626	<0.0001
TAZ		A transcriptional co-repressor of PPARγ that attenuates adipogenesis	−0.738	<0.0001
WNT3A		Inhibitor of adipogenesis	0.497	0.003
VDR		Unliganded VDR can activate adipogenesis through increased lipid accumulation	−0.532	0.001
TCF7L2		Adipose specific knockout of Tcf7l2 leads to adipocyte hypertrophy and impaired lipolysis	−0.397	0.022
TSC22D3		Inhibitor of the adipogenic master regulator PPARγ	−0.657	<0.0001
NCOA2	Anti-BAT tissue	Required with NCOA1 to control energy balance between white and brown adipose tissues	−0.704	<0.0001
TWIST1		Inhibit UCP-1 in brown adipocytes	−0.385	0.027
RB1		Molecular switch determining white vs. brown adipogenesis	−0.703	<0.0001
NR0B2		A negative regulator of PGC-1α expression in brown adipocytes	0.537	0.001
GATA2	Anti-white adipose tissue	Suppressor of adipocyte differentiation	−0.558	0.001
KLF2		Negative regulator of adipogenesis	−0.568	0.001
KLF3		Induction of Wnt10b and KLF3 decrease PPARγ expression	−0.567	0.001
AXIN1	Pro-adipogenesis	Promoter of adipocyte differentiation	−0.799	<0.0001
CCND1			−0.458	0.007
CDK4			−0.817	<0.0001
CEBPA			−0.579	0.0001
CEBPB			−0.553	0.001
CEBPD			−0.369	0.034
E2F1			−0.515	0.002
FGF1			−0.450	0.009
FGF2			−0.670	<0.0001
IRS2			0.560	0.001
SFRP1			−0.354	0.043
SLC2A4			−0.369	0.034
LMNA			−0.810	<0.0001
PPARG			−0.506	0.003
RXRA		Implicated in the development of dysfunctional white adipose tissue	−0.700	<0.0001
CREB1	Pro-brown adipose tissue	Involved in the transcriptional regulation of brown adipogenesis	−0.693	<0.0001
DIO2		Involved in the transcriptional regulation of brown adipogenesis	−0.791	<0.0001
INSR		Regulates metabolism in brown adipose tissue	−0.411	0.017
IRS1		Involved in the differentiation of brown adipocytes	−0.449	0.009
NRF1		Involved in brown adipose tissue thermogenic adaptation	−0.775	<0.0001
MAPK14		Controls brown adipocyte differentiation	−0.715	<0.0001
PPARA		Regulators of brown adipogenesis/metabolism	−0.619	0.0001
PPARD			−0.740	<0.0001
PPARGC1B			−0.389	0.025
PRDM16			−0.525	0.002
WNT5A			−0.458	0.007
SRC		Controls brown fat thermogenesis	−0.412	0.017
SIRT3			−0.745	<0.0001

Continued

Table 2 *Continued*

b) Adipogenis genes and glucose-dependent insulinotropic peptide receptor (GIPR)

Genes	Family group	Function	Correlation coefficient	*P* value
BMP7	Pro-white adipose tissue	Promoters of white adipogenesis	−0.521	0.002
BMP2			−0.512	0.002
BMP4			−0.709	<0.0001
CEBPA		Required for differentiation of white adipocytes	−0.579	0.0004

Glucagon receptor (GCGR)

c) Fatty acid metabolism genes and glucagon receptor (GCGR)

Genes	Family group	Function	Correlation coefficient	*P* value
ACADSB	Acyl-CoA dehydrohgenases	It has the greatest activity towards the short branched chain acyl-CoA derivative	0.3753	0.0314
EHHADH		One of the four enzymes of the peroxisomal beta-oxidation pathway	−0.3693	0.0344
ACADL		One of the four enzymes that catalyse the initial step of mitochondrial beta-oxidation of straight-chain fatty acid	0.3815	0.0285
ACAD10		Involved in the beta-oxidation of fatty acids in mitochondria	0.4278	0.00130
ACSBG1	Acyl-CoA synthetases	Activate long- and medium-chain FFA for oxidation	0.3850	0.0269
ACSBG2			0.8244	<0.0001
ACSL6			0.3643	0.0371
ACSM3			0.7245	<0.0001
ACSM4			0.8426	<0.0001
ACOT12	Acyl-CoA thioesterases	Regulate FFA oxidation in mitochondria and peroxisomes	0.8235	<0.0001
ACOT6			0.8001	<0.0001
CROT	Fatty acid transport	Transport FFA into mitochondria for oxidation	−0.4649	0.0064
SLC27A3		Facilitate FFA transport across membranes	0.6557	<0.0001
FABP1		Cytoplasmatic FFA transport proteins	0.7942	<0.0001
FABP2			0.8285	<0.0001
FABP12			0.7868	<0.0001
FABP6			0.7830	<0.0001
PRKAA1	Fatty acid biosynthesis regulation	AMP-activated, alpha 2 catalytic subunit: inhibits ATP-consuming biosynthetic pathways	0.5241	0.0017
PRKAA2		AMP-activated, alpha 1 catalytic subunit: inhibits ATP-consuming biosynthetic pathways	0.6581	<0.0001
PRKACA		Catalytic subunits of protein kinase A: promotes fat utilization	0.8138	<0.0001
PRKACB		Catalytic subunit of cAMP (cyclic AMP)-dependent protein kinase	0.5291	0.0015
PRKAG3		Regulatory G1 subunit of AMP-activated protein kinase: inhibits ATP-consuming biosynthetic pathways	0.4442	0.0096
PRKAG2		Regulatory G2 subunit of AMP-activated protein kinase: inhibits ATP-consuming biosynthetic pathways	0.9809	<0.0287
GK	Triacylglycerol metabolism	Esterification of FFA with reduced FFA efflux	−0.5013	0.0030
HMGCS2	Ketogenesis and ketone body metabolism	Involved in the synthesis and degradation of ketone bodies	0.8085	<0.0001
OXCT2		Involeved in ketone body catabolism	0.7908	<0.0001

d) Adipogenesis genes and glucagon receptor (GCGR)

Genes	Family group	Function	Correlation coefficient	*P* value
RETN	Adipokines and hormones	A feedback regulator of adipogenesis	0.6892	<0.0001
ADIG		Adipocyte-specific protein that plays a role in adipocyte differentiation	0.8425	<0.0001
HES1	Anti-adipogenesis	Inhibits adipocyte differentiation	−0.4395	0.0105
DLK1			0.7219	<0.0001
SHH			0.8773	<0.0001
TCF7L2			0.6788	<0.0001

Continued

Table 2 *Continued*

d) Adipogenesis genes and glucagon receptor (GCGR)				
Genes	**Family group**	**Function**	**Correlation coefficient**	***P* value**
WNT1			0.8179	<0.0001
WNT3A			0.8289	<0.0001
RUNX1T1			−0.4275	0.0131
NCOA2	Anti-brown adipose tissue	Required with NCOA1 to control energy balance between white and brown adipose tissues	0.5221	0.0018
WNT10B		It blocks brown adipose tissue development and expression of UCP-1.	0.7189	<0.0001
RB1		Molecular switch determining white vs. brown adipogenesis	0.5211	0.0019
NR0B2		A negative regulator of PGC-1α expression in brown adipocytes	0.8780	<0.0001
GATA2	Anti-white adipose tissue	Suppressor of adipocyte differentiation	0.6982	<0.0001
GATA3		Involved in white browning	0.4522	0.0082
KLF2		Negative regulator of adipogenesis	0.4318	0.0121
KLF3		Induction of Wnt10b and KLF3 decrease PPARγ expression	0.6765	<0.0001
IRS2	Pro-adipogenesis	Promoter of adipocyte differentiation	0.6517	<0.0001
CEBPB			0.4369	0.0110
FGF1			0.3830	0.0278
SFRP5			0.6130	0.0001
WNT5B			0.3917	0.0242
CREB1	Pro-brown adipose tissue	Involved in the transcriptional regulation of brown adipogenesis	−0.4830	0.0044
FOXC2			0.8443	<0.0001
MAPK14			0.3981	0.0218
PPARA		Regulators of brown adipogenesis/metabolism	0.7383	<0.0001
PPARD			0.8281	<0.0001
PPARGC1A			0.6832	<0.0001
UCP-1			0.5635	0.0006
PRDM16			0.7189	<0.0001
SRC		Controls brown fat thermogenesis	0.4412	0.0102
BMP7	Pro-white adipose tissue	Promoters of white adipogenesis	0.5625	<0.0007
KLF15			0.6768	<0.0001

Table reports existing positive and negative correlations of glucose-dependent insulinotropic peptide receptor (GIPR) and glucagon receptor (GCGR) with genes involved in fatty acid metabolism and adipogenesis.
Spearman correlation coefficients and corresponding *P* value are reported.

demonstrations of the peptide receptors in EAT macrophages, thus, can be of interest to understand how the GIP/GLP-1 and GLP-1/GIP/GCG associations could exert their potential immunological effects in adipose and cardiac tissues, given for example the conflicting results regarding GIP's pro- and anti-inflammatory effects in atherosclerotic plaque formation.[30–32] This evidence could open a new perspective on these therapies' long-term effects, suggesting that the activation of EAT GIP and GCG receptors could play some roles in epicardial fat inflammation, ectopic lipid accumulation, and thermogenesis. Similarly, our findings of GLP-1R and GIPR expression in EAT vessels may add relevant information on novel drug targets for cardiovascular therapy. As a result of treatment with liraglutide, a widely used GLP-1R agonist, for 26 weeks, the uptake of [^{64}Cu]Cu-DOTATATE—measured by positron emission tomography (PET) as a marker of coronary inflammation—in the coronary arteries was significantly reduced compared to the placebo-treated group.[33] The long-acting GLP-1R agonist semaglutide reduces vascular inflammation investigated by PET in a rabbit model of advanced atherosclerosis.[34] Moreover, tirzepatide, the dual GIP/GLP-1 receptor agonist, has been found to improve cardiovascular risk biomarkers in patients with type 2 diabetes, including circulating levels of biomarkers of systemic inflammation, cytokines, adhesion molecules, and the cardiomyocyte stress marker *N*-terminal-pro hormone B-type natriuretic peptide suggesting beneficial effects on immune and vascular cells.[35] The relevance of these results is further strengthened by the observation that GIPR and GCGR are also widely expressed in the myocardium.[36]

We also found that EAT GIPR is associated with genes involved in FFA pathways, including those regulating oxidation, biosynthesis, and transport. Interestingly, GIPR was related to acyl coenzyme A (Acyl-CoA) synthetases and Acyl-CoA thioesterases genes involved in medium- and long-chain fatty acid oxidation and FFA oxidation in the mitochondria and peroxisomes. Glucose-dependent insulinotropic polypeptide receptor is additionally correlated with the catalytic subunit of kinase A involved in fat utilization. On the other hand, GIPR gene was also associated with genes involved in FFA synthesis rather than utilization, adipogenesis, and white-to-brown fat differentiation.

Given the myocardium relies mostly on FFAs as fuel and is in direct contiguity with EAT, these findings may have particular relevance for the modulation of the myocardial activity and performance.[1,2,6]

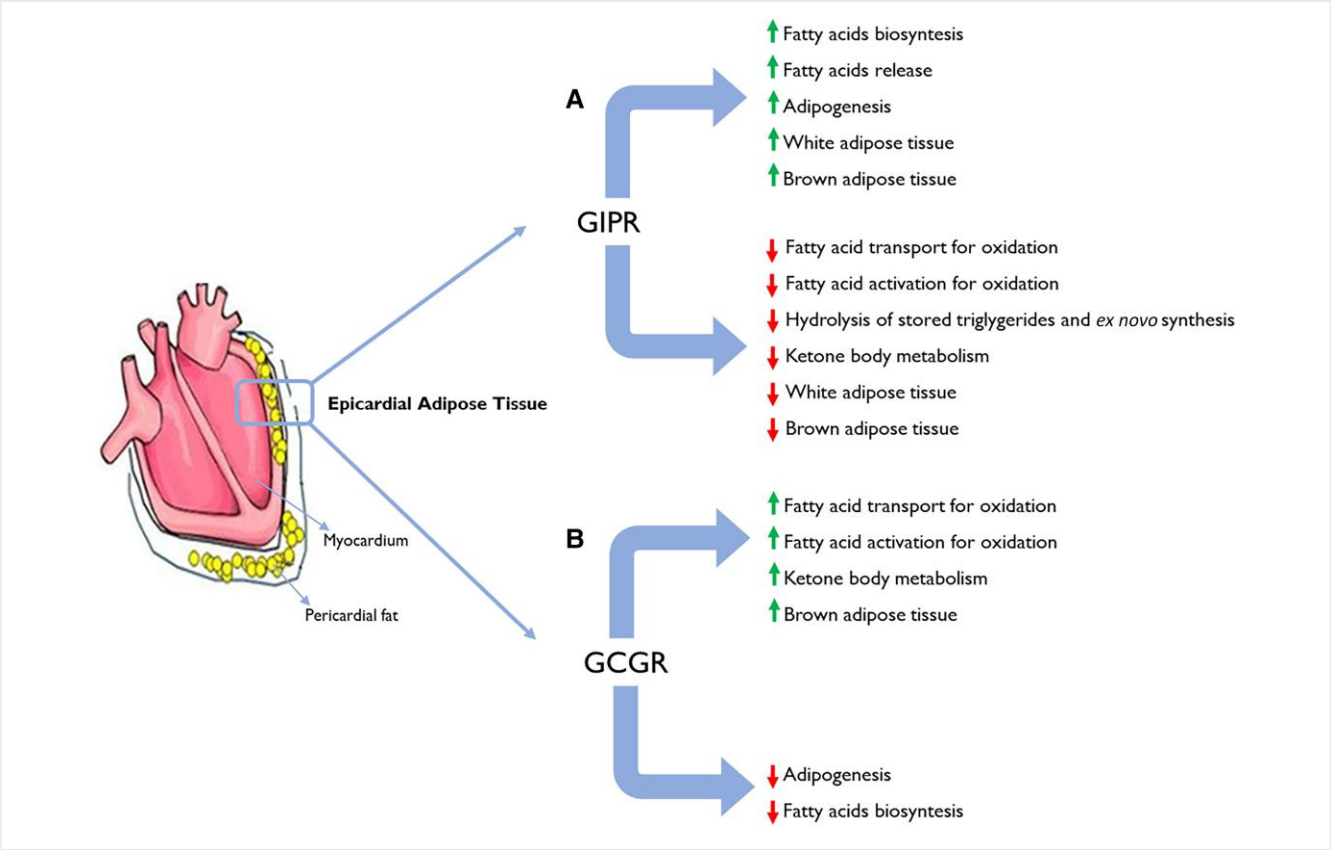

Figure 4 Metabolic pathways associated to GIPR and GCGR expression levels in EAT samples. (*A*) resumes metabolic changes associated to glucose-dependent insulinotropic peptide receptor (GIPR) expression in epicardial adipose tissue (EAT) samples. (*B*) resumes metabolic changes associated to glucagon receptor (GCGR) expression in EAT samples.

We previously showed that EAT GLP-1 receptor expression was directly correlated with genes promoting FFA oxidation and white-to-brown adipocyte differentiation and inversely with pro-adipogenic genes, suggesting that targeting EAT GLP-1R by GLP-1R analogues may reduce local adipogenesis, improve fat utilization, and induce brown fat differentiation.[15] Interestingly, we found that EAT GIPR and GPL-1R were correlated with each other, although GIPR expression was 1107-fold higher than GLP-1R.

The GIP receptor presence in EAT adipocytes may further strengthen these findings. Glucose-dependent insulinotropic polypeptide receptor is expressed in visceral and subcutaneous fat,[37,38] and its stimulation directly promotes triglyceride storage in adipocytes. The GIP-dependent insulin release indirectly promotes triglyceride accumulation in fat cells.[39] Higher levels of GIPR gene expression were found in visceral fat tissue compared to subcutaneous fat tissue.[38] It has been suggested that GIP/GIPR axis may have an anti-atherosclerotic activity, reducing oxidative stress in human endothelial cells and inflammatory cytokine release in visceral adipose tissue.[30,31] Although we did not find any differences in EAT GIPR expression between patients with or without abdominal obesity (determined by waist circumference cut off points), our data showed that EAT GIPR gene expression levels were higher in patients with higher visceral adiposity (determined by the ultrasound-measured EAT thickness, a more accurate marker of visceral fat than waist circumference).[40]

It has been reported that GIPR expression could be down-regulated with hyperglycaemia and insulin resistance.[38,41] However, in our study, both CABGs and controls showed excellent glucose control and similar insulin sensitivity with no difference in EAT GIPR expression.

In this study, we also found, for the first time, that EAT expresses the GCGR gene and protein. Notably, EAT GCGR expression was positively correlated with EAT genes such as forkhead box protein C2 (FOXC2), GATA binding protein 3 (GATA3), PPARGC1A, SRC, and UCP-1, all encoding for brown fat activation or white-to-brown fat differentiation. Moreover, WNT1, a gene encoding for factors reducing adipogenesis, was also significantly related to EAT GCGR expression. Notably, a positive correlation has been found between EAT GCGR and GIPR and between GCGR and GLP-1R. In addition, we found that EAT GCGR level of expression was 5.17-fold higher than GLP-1R and 203-fold lower than GIPR.

The significance and implication of these findings are still unclear. Glucagon receptor agonists increase energy expenditure in adult human to a magnitude sufficient for inducing a negative energy balance.[42] In preclinical studies, the Glucagon-GCGR system affects key metabolically relevant organs to boost whole-body thermogenic capacity and protect from obesity.[19,20] The activation of the GCGR has been shown to augment both the magnitude and duration of weight loss, achieved by either selective GLP-1R or dual GIPR/GLP-1R agonism in rodents.[42]

Further studies evaluating the safety and efficacy of GLP-1R/GIPR/GCGR triagonists are warranted.

Limitations

Our study has some limitations. First, we did not collect subcutaneous adipose tissue (SAT) during cardiac surgery to explore potential differences among fat depots. However, because previous studies showed

that EAT and SAT have a very distinctive transcriptome and secretome, including GLP-1R (not detected in SAT), we may also observe similar differences in GIPR and GCGR.[5,14] Second, this study did not include inflammatory genes whose EAT is highly enriched because we intentionally focused on EAT genes involved in adipogenesis and FFA metabolism. Third, echocardiography was used to measure EAT thickness rather than volume. Fourth, our analyses focused on the expression of receptors in EAT as this peculiar fat depot not only is composed of adipocytes but also contains nerve tissues, inflammatory, stromovascular, and immune cells. Future interventional studies looking at the effects of selective agonists of G-protein–coupled receptor agonists on epicardial fat transcriptome are warranted. Fifth, our study had a cross-sectional design; therefore, we could study only associations and no causal mechanisms.

Conclusion

In conclusion, this is the first time human that EAT has been found to express GIPR and GCGR genes and proteins, suggesting novel pathways and mechanisms of action for selective agonists of these G-protein–coupled receptors.

Given the emerging use of the GLP-1R agonists, dual GIPR/GLP-1R agonists, and GLP-1R/GIPR/GCGR triagonists, we believe that pharmacologically targeting and potentially modulating organ-specific fat depots through GLP-1, GIP, and GCG receptors may produce beneficial cardiovascular and metabolic effects that would go beyond the glucose control and weight loss.

Authors' contributions

Alexis Elias Malavazos, Gianluca Iacobellis, and Elena Dozio contributed to the conception or design of the work. All authors contributed to the acquisition, analysis, or interpretation of data for the work, drafted, and critically revised the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

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Supplementary material

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Conflict of interest: None declared.

Data availability

The data underlying this article are available in the article and in its online Supplementary material.

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