1. Introduction

EAT is the visceral fat of the heart with unique anatomy, functionality and transcriptome (1). EAT has recently emerged as an important cardiovascular risk factor and therapeutic target (2). EAT is highly enriched with genes involved in inflammation, coagulation and immune signaling, when compared to subcutaneous fat (1). Due to its peculiar contiguity with the myocardium, EAT pro-inflammatory secretosome may directly affect the heart and coronary artery metabolism, and therefore cause atherosclerosis, as previously described by our group (1-8). Glucagon-like peptide 1 analogs (GLP-1A) have recently shown important cardiovascular beneficial effects that go beyond their antidiabetic actions (9-11). We recently reported a substantial reduction (by 36%) of the ultrasound measured EAT thickness in diabetic and obese patients receiving additional liraglutide, a widely used GLP-1A, to metformin, independently of the overall weight loss and improved glucose control. A milder, yet noticeable (-13%), reduction of EAT thickness was recently observed with either liraglutide or exenatide in a smaller group of patients with type 2 diabetes (DM) (12). To corroborate the hypothesis of a targeting GLP-1A effect, we performed a RNA-sequencing (RNA-seq) analysis on EAT collected during cardiac surgery and found, for the first time, that human EAT expresses GLP-1 receptor (*GLP-1R*) and *GPL-2R* (12). All together this suggests that the GLP-1A cardioprotective effects may be mediated by improvements in EAT functions and transcriptome. Nevertheless, the genetic and metabolic changes behind the beneficial effects of GLP-1A on EAT are unknown. Although its role is not yet understood, GLP-2 can also exert beneficial effects on adipose tissue metabolism (13-16), but its correlation with EAT transcriptome is unexplored.

Hence, in this study we sought to analyze whether EAT transcriptome regulating FA metabolism and adipogenesis is related to *GLP-1R* and *GLP-2R* gene expression and GLP-1 and GLP-2 plasma levels in patients with coronary artery disease (CAD).

2. Materials and Methods

2.1 Study population

A total of 47 patients with CAD and 25 healthy volunteers (CTR) were enrolled in the study. CAD patients were recruited among those who underwent elective coronary angiography at the IRCCS

Policlinico San Donato between October 2011 and June 2013. Patients with clinically and angiographically detected CAD were included in the study. We excluded patients with the following criteria: age ≤18 years, acute myocardial infarction in the previous month, end-stage heart failure, valve diseases or other heart diseases different from CAD, malignant diseases, major abdominal surgery in the previous six months, renal and liver diseases, chronic inflammatory diseases, more than 3% change in body weight in the previous three months, missing or incomplete clinical history and data, and current use of GLP-1A and dipeptidyl peptidase 4 (DPP4) inhibitors. Among the 47 CAD patients, 17 required elective coronary artery bypass grafting (CABG), an elective open heart procedure in hemodynamically stable patients taking their standard cardiac treatments and under the care of the cardiologist. EAT samples were collected just from these 17 patients during surgery. Blood samples for plasma quantification of GLP-1 and GLP-2 levels were obtained from the total 47 CAD patients and the 25 CTR subjects. Written informed consent was obtained from all participants. The study was approved by the local ethics committee (ASL Milano Due, protocol 2516) and conducted in accordance with the Declaration of Helsinki, as revised in 2013, and Good Clinical Practice guidelines.

2.2 Biochemical parameters

Blood samples were collected after an overnight fasting, into pyrogen-free EDTA tubes or in tubes for serum collection. EDTA plasma samples for non-routine assays were obtained after centrifugation at 1200 g for 15 min and immediately stored at -20°C until subsequent analyses. Cobas 6000 analyzer and commercial kits (Roche Diagnostics, Milan, Italy) were used for the quantification of routine biochemical parameters, as previously reported (2,5,17). LDL-cholesterol was calculated with the Friedewald formula. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: HOMA-IR = fasting insulin [μU/mL] x fasting glucose [mmol/L]/22.5.

2.3 Anthropometric measures

Weight, height and waist circumference (WC) were directly measured at hospital admission. Weight and height were recorded to the nearest 0.1 kg and 0.5 cm using standard scales and stadiometers. WC was measured using a flexible tape. Body mass index (BMI) and waist-to-height ratio (WHtR) were then calculated as weight (kg)/height^2 (m^2) and WC (cm)/height (cm), respectively. As defined by WHO, patients were classified as normal weight (BMI 18.5-24.9 kg/m2), overweight (BMI 25.0-29.9 kg/m2) and obese (BMI ≥ 30.0 kg/m2). A WHtR ≥ 0.5 indicated central obesity (18).

2.4 EAT thickness measurement

All patients underwent standard echocardiography using commercially available equipment (Vingmed-System Five; General Electric, Horten, Norway). EAT thickness was measured according to the method first described and validated by Iacobellis et al. (8-9). Briefly, EAT was identified as the echo-free space between the outer wall of the myocardium and the visceral layer of pericardium. EAT thickness was measured perpendicularly on the free wall of the right ventricle at end-systole in three cardiac cycles. The parasternal long-axis view allowed for the most accurate measurement of EAT on the right ventricle, with optimal cursor beam orientation in each view. Maximum EAT thickness was measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aortic annulus, used as the anatomical landmark for this view. The average value of three cardiac cycles was calculated and used for analysis.

2.5 EAT 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.

2.6 RNA extraction and microarray analysis

Total RNA was extracted from tissue with the RNeasy Lipid Tissue Kit according to the manufacturer's procedure (Qiagen). RNA 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). Gene expression was analysed by a one-color microarray platform (Agilent): 50 ng of total RNA were labeled with Cy3 using the Agilent LowInput Quick-Amp Labeling kit-1 color, according to the manufacturer's directions. cRNA was purified with the RNeasy Mini Kit (Qiagen) and the amount and labeling efficiency were measured with NanoDrop.

For hybridization we used an Agilent Gene Expression Hybridization Kit and scanned with the Agilent G2565CA Microarray Scanner System. Data were processed using Agilent Feature Extraction Software (10.7) with the single-color gene expression protocol, and raw data were analyzed 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 based on the SAM algorithm by Tusher (19). -Fold changes were calculated from normalized data.

2.7 GLP-1 and GLP-2 enzyme-linked immunosorbent assays (ELISA)

Circulating levels of total GLP-1 and GLP-2 were quantified on EDTA-plasma samples according to the manufacturer's directions with the following ELISA assays: EZGLP1T-36K for GLP-1 and EZGLP2-37K for GLP-2 (Merck S.p.A, Milan, Italy). The minimum detectable dose was 1.5 pM for s GLP-1 and 0.3 ng/mL for GLP-2. The maximum intra- and inter-assay coefficients of variation were respectively 2% and <12% for GLP-1 and 9.1% and 11.5% for GLP-2. The GloMax®-Multi Microplate Multimode Reader was used for photometric measurements (Promega, Milan, Italy).

2.8 Statistical analysis

Quantitative variables are expressed as median and 25th-75th percentiles or mean ± SD. Qualitative variables are summarized as numbers and percentages. The normality of data distribution was assessed with the Kolmogorov-Smirnoff test. Comparison between two groups was performed by T-test or Mann-Whitney tests for continuous variables. For group-wise comparison (three groups), ANOVA or Kruskal-Wallis tests followed by Bonferroni or Dunns tests were used, as appropriate. Fisher's exact test was used for nominal variables. Relations between parameters were examined with

the Spearman correlation test. Data were analyzed using GraphPad Prism 5.0 biochemical statistical package (GraphPad Software, San Diego, CA). A p value <0.05 was considered significant.

3. Results

3.1 Patient characteristics

The main demographic, anthropometric, clinical and biochemical characteristics of the 47 CAD patients enrolled in the study are shown in **Table 1**. The mean age was 65.40 ± 9.49 years and the majority of patients were males. According to BMI, nearly one fifth of the patients were obese and almost two thirds overweight. WHtR indicated that 43 patients (91%) had a central obesity. EAT thickness ranged from 3 to 12 mm (mean 7.77 ± 2.32 mm). Fifteen patients had a diagnosis of DM. Among these, 12 were under antidiabetic drugs and displayed a good glycemic control (fasting glucose 95.69 ± 16.42 mg/dL, HbA1c 6.02 ± 1.18 %). Of the 47 CAD patients, 17 underwent CABG. The main features of this subgroup are also shown in **Table 1.** No statistical differences have been observed between CAD and CABG subgroup, except for HbA1c that was lower in CABG compared to CAD (4.69 ± 1.42% *vs*. 5.64 ± 1.50%). DM was present just in 1 patient in CABG group. Although BMI did not identify any patient as obese, 15 patients (88.24%) had a central obesity, according to WHtR $\geq$ 0.5. The mean EAT thickness value in CABG subgroup was the same observed in the whole CAD group (mean 7.24 ± 2.36 mm, median 8). Compared to healthy subjects, CAD patients as well as CABG subgroup displayed higher percentages of cardiovascular risk factors and use of drugs (**Table 1**).

3.2 GLP1-R and GLP2-R expression in EAT

GLP1-R and *GLP2-R* expression in EAT was examined at gene level. Both receptors were detected and GLP2-R levels (149.10 arbitrary unit, a.u.) were higher than GLP1-R (2.61 a.u.) (p <0.0001).

3.3 Correlation of GLP1-R with genes involved in FA metabolism and adipogenesis

We investigated 84 genes involved in FA metabolism and 84 involved in adipogenesis in EAT, and correlated them with *GLP1-R* levels. Twenty-two genes related to FA metabolism were positively

correlated with GLP1-R and 6 negatively. Out of the 84 genes involved in adipogenesis, 17 were directly correlated with *GPL1-R* expression and 9 inversely. Names, functions and correlation parameters of genes related to FA and adipogenesis are reported in detail in **Table 2a** and **2b**, respectively. EAT *GLP-1R* was positively correlated with genes increasing FA oxidation, switching-on beta-oxidation, decreasing FA release into the coronary circulation, reducing adipogenesis and regulating the differentiation from white-to-brown fat. **Figure 1a** resumes the pathophysiological significance of the observed correlations.

3.4 Correlation of GLP2-R with genes involved in FA metabolism and adipogenesis

Correlation analyses were also done for *GLP-2R*. *GLP2-R* was correlated, inversely, just with 2 genes related to FA metabolism and directly with 4 genes involved in adipogenesis. Names, functions and correlation parameters of FA and adipogenesis genes are reported in detail in **Table 3a** and **3b,** respectively. According to these correlations, EAT *GLP-2R* is related to genes promoting adipogenesis, reducing FA transport and activation for mitochondrial beta-oxidation. **Figure 1b** resumes the pathophysiological significance of the observed correlations.

3.5 Plasma levels of GLP-1 and GLP-2

GLP-1 and GLP-2 plasma levels were measured in CAD patients and in a group of 25 CTR subjects, whose main biochemical features are indicated in **Table 1**. Both GLP-1 and GLP-2 were higher in CAD than CTR ($p < 0.0001$ and $p < 0.001$, respectively) **(Figure 1, panel c and d)**. EAT thickness was also higher in CAD than CTR (7.88 ± 2.39 mm *vs.* 3.69 ± 1.78 mm, $p < 0.0001$). After classification according to the EAT median thickness (8 mm), both GLP-1 and GLP-2 levels were higher in group with the greater ultrasound-measured EAT thickness ($p < 0.05$) **(Figure 1c** and **d,** respectively**)**. Correlation analyses of GLP-1 and GLP-2 plasma levels with *GLP-1R* and *GLP-2R* expression in EAT did not reach the statistical significance ($p < 0.05$ for all).

4. Discussion

We believe this study provides findings of novelty and interest. For the first time we found that EAT *GLP-1R* is associated with up-regulated genes involved in free FA oxidation and white-to-brown adipocyte differentiation, and decreased adipogenesis. On the contrary up-regulation of EAT *GLP2-R* was correlated with genes involved in adipogenesis and lipid synthesis, and reduced FA activation and transport for mitochondrial beta-oxidation. These data suggest that specific metabolic changes occurring in EAT in CAD, which contribute to increase EAT amount and therefore EAT detrimental effects on coronary vessels and myocardium, are also strictly related to changes in the local expression of GLP receptors. Therefore, targeting *GLP1-R* could really represent an intriguing strategy to reverse metabolic derangement of EAT. Notably, recent insights describing a reduction of EAT amount in patients under GLP-1A therapy seem to strongly sustain our hypothesis.

It has been reported that GLP-1 may play a role in different tissues and *GLP-1R* stimulation may promote not only insulin secretion by pancreatic beta-cells, but also vascular relaxation, down-regulation of pro-atherosclerotic factors in endothelial cells and hepatic lipid oxidation (20-23). Although the presence of *GLP-1R* in isolated human and rat adipocytes has been reported since the 1990s (24-26), the implications of GLP-1 signaling in adipose tissue are still poorly understood. Iacobellis et al. first demonstrated that human EAT expresses *GLP-1R* at both gene and protein level (15). Clinically, liraglutide, a GLP-1A, induced a substantial decrease of ultrasound-measured EAT thickness in DM and obese patients (16). Although the shrinking effect of liraglutide on EAT thickness was higher and not proportional with changes in BMI, a role of liraglutide-induced weight loss in reducing EAT could not be completely ruled out (26,27). Also, the mechanisms explaining the significant decrease (by almost 40%) of EAT on liraglutide were not clear. So, to our knowledge, this is the first study addressing and showing the relationship between EAT, *GLP-1R* and related adipogenic and metabolic transcriptome. In fact, we found that EAT genes involved in FA metabolism, such as those encoding for Acyl-CoA Thioesterases and Acyl-CoA Synthetases, showed a positive correlation with EAT *GLP-1R*. These genes promote FA oxidation into mithocondria and peroxisomes, FA esterification and FA transfer across membranes. Remarkably, EAT *GLP-1R* was positively correlated with EAT genes such as *FOXC2 GATA3, PPARGC1A, SRC* and *UCP1,* all

encoding for brown fat activation or white-to-brown fat differentiation. *WNT1*, a gene encoding for factors reducing adipogenesis, was also significantly related to EAT *GLP-1R*. Based on these findings, it is tempting to speculate that GLP-1A activation of EAT *GLP-1R* can induce a cascade of events leading to a better lipid energy utilization and local fat reduction. As EAT lies in direct contiguity with the myocardium and shares the same microcirculation (1), it is plausible to hypothesize that EAT *GLP-1R* stimulation could reduce excessive fat influx into the myocardium. The lack of fascial barrier and shared microcirculation allows for a bi-directional crosstalk through paracrine and vasocrine pathways (1). It is interesting to report as cardiomyocytes also express *GLP-1R*, as recently discovered (28). GLP-1 agonism may therefore target both cardiac cells and fat. EAT *GLP-1R* expression seems to be down-regulated or at least lower than *GLP-2R* expression in our samples collected from CAD patients. Some suppression of gene enrichment in the EAT of CAD subjects has been previously reported (4) and attributed to the relative inactivity or down-regulation of robust cellular activities of this tissue in the setting of severe and chronic CAD. Moreover, previous studies indicated that obesity and DM were associated to a decreased expression of *GLP-1R* in human peritoneal adipose tissues, pancreatic beta-cells and human arteries and chronic gluco-lipotoxicity was likely to reduce its expression (29). Therefore, since CAD patients had a greater ultrasound-measured EAT thickness, lower levels of *GLP-1R* may be strongly related to the increased EAT amount too. In our study we have also measured total GLP-1 plasma levels to evaluate any potential relationship with EAT *GLP-1R* expression. In fact, we cannot exclude that any changes occurring systemically may also influence the local expression of the receptor. Unfortunately, we could quantify only total GLP-1 levels, but not its active form because samples have been long-term stored without the addition of a DPP4 inhibitor that prevents the degradation of the active form. Our observation that GLP-1 levels are increased in CAD and, among CAD, in those with increased ultrasound-measured EAT thickness might indicate both the activation of a potential counter-regulatory mechanism that try to compensate a reduction in the active form as well as an attempt to improve EAT metabolism. Once again, these data seem to confirm an alteration of the GLP-1 system in CAD and reinforce the idea that the use of active GLP-1A may improve EAT function.

224 Besides GLP-1, our study also focused on GLP-2. GLP-2 is mainly an intestinotrophic factor involved
225 in maintaining the integrity and morphology of the intestinal mucosa, increasing its absorptive surface
226 and properties (15). Although GLP-2 does not influence insulin secretion (30,31), a role for
227 endogenous GLP-2 in improving glucose metabolic disorders induced by a high-fat diet has been
228 proposed (13,32). Little is known about GLP-2R-mediated physiological effects. Only recently, *GLP-*
229 *2R* expression has been reported in EAT and no data are available about other adipose tissue
230 compartments (12). Evidence from tissue-specific *GLP-2R* KO mice indicated a physiological role in
231 the control of food intake and glucose homeostasis (33). In obesity, elevated *GLP-2R* levels have been
232 detected in gastric chief cells as a regulatory response associated with nutrient status (34). Our
233 findings confirmed that EAT expresses *GLP-2R* and show, for the first time, that its levels are related
234 to genes promoting adipogenesis and fat accumulation. Although the clinical significance of this
235 observation remains to be established, previous insights on the role of *GLP-2R* prompted us to
236 consider that its expression is up-regulated or at least higher than *GLP-1R* as a potential compensatory
237 mechanism related to EAT expansion, more than a direct cause of adipogenesis and fat deposition. Of
238 course, the role of *GLP-2R* needs further investigation.
239
240 Our study have some limitations. First, we did not collect subcutaneous fat (SAT) during cardiac
241 surgery to explore any potential difference among various fat depots and due to the amount of EAT
242 isolated during surgery we could evaluate only EAT transcriptome, not the corresponding proteome.
243 However, as previous studies already showed the differences in the transcriptome between EAT and
244 SAT (8,12,35), we can expect to observe differences also about *GLP-1R* and *GLP-2R* functions.
245 Second, we had no surgical control group to compare *GLP-1R* and *GLP-2R* related genes between
246 CAD and CTR. However, differences in EAT genetic profile between CAD and no-CAD subjects
247 have been evaluated and reported before by our group and others (2,7,8,35-37). Third, inflammatory
248 genes of whose EAT is highly enriched, as previously reported by our group (5,38), were not included
249 in this study because we intentionally focused our attention to EAT genes involved in adipogenesis
250 and FA metabolism. Fourth, echocardiography was used to measure EAT thickness, as reliable and
251 not invasive methodology. Future studies using CT scan assessment of EAT volume and peri-

coronary EAT are warranted. Fifth, our study has a cross sectional design, therefore only associations were investigated and no causal mechanisms were proved.

5. Conclusions.

EAT is a potential target of the effects of GLP-1 and GLP-2. EAT *GLP-1R* expression is associated with EAT genes involved in FA oxidation and white-to-brown fat differentiation. GLP-1A may therefore target EAT *GLP-1R* and reduce local adipogenesis, improve fat utilization and induce brown fat differentiation. As EAT lies in direct contiguity to the myocardium and coronary arteries, the beneficial effects of *GLP-1R* activation may extent to the heart. The increased levels of circulating GLP-1 and GLP-2 and EAT *GLP-2R* may be compensatory mechanisms related to CAD and also EAT expansion, but the meaning of these observations needs to be further investigated.

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Figure legends

Figure 1. Metabolic pathways associated to *GLP-1R* and *GLP-2R* levels in EAT from CAD patients. Panel a resumes metabolic changes associated to *GLP-1R* expression in EAT. **Panel b** resumes metabolic changes associated to *GLP-2R* expression in EAT.

Figure 2. Plasma levels of GLP-1 and GLP-2. GLP-1 and GLP-2 plasma levels were quantified in healthy subjects (CTR) and in CAD patients. **Panel a** shows GLP-1 levels in CTR, all CAD patients and in CAD patients classified according to the median EAT thickness (8 mm). **Panel b** shows GLP-2 levels in CTR, all CAD patients and in CAD patients divided according to the median EAT thickness. ** $p < 0.001$ and *** $p < 0.0001$ *vs.* CTR; ° $p < 0.05$ *vs.* EAT <8 mm.

Table 1.

Demographic, anthropometric, clinical and biochemical characteristics of patients and healthy subjects included in the study.

	CAD (n = 47)	CABG (n = 17)	Healthy (n = 25)
Age (years)	65.40 ± 9.49, 65.00 (59.00-73.00)[a]	67.12 ± 10.75, 68.00 (56.50-79.00)[a]	33.56 ± 7.48, 33.00 (29.00-38.00)
Male gender (n, %)	43, 91.45%[a]	15, 88.24%[a]	0
BMI (kg/m^2)	27.82 ± 4.25, 27.27 (25.40-29.13)	27.69 ± 5.65, 26.54 (25.33-28.37)	27.47 ± 4.75, 26.98 (22.90-32.46)
WC (cm)	104.80 ± 13.08, 104.00 (98.75-110.00)[a]	104.50 ± 17.49, 102.00 (94.50-114.00)[b]	85.50 ± 12.53, 83.00 (74.00-97.25)
WHtR	0.62 ± 0.08, 0.61 (0.57-0.67)[a]	0.62 ± 0.09, 0.60 (0.55-0.66)[c]	0.51 ± 0.13, 0.54(0.46-0.60)
EAT thickness (mm)	7.77 ± 2.32, 8.00 (6.00-9.00)[a]	7.24 ± 2.36, 7.00 (5.50-9.00)[a]	3.69 ± 1.78, 3.50 (2.00-5.50)
Fasting glucose (mg/dl)	104.80 ± 47.83, 88.00 (79.00-114.00)	106.30 ±35.61, 95.00 (79.50-121.50)	84.20 ± 7.47, 85.00 (77.00-91.50)
Fasting insulin (microU/ml)	9.52 ± 7.55, 7.54 (5.07-11.98)	10.64 ± 10.70, 6.91 (4.03-12.89)	10.64 ± 5.14, 9.55 (7.47-12.82)
HbA1c (%)	5.64 ± 1.50, 5.48 (5.00-6.31)[d]	4.69 ± 1.42, 4.43 (3.45-5.90)	-
HOMA-IR	2.34 ± 2.04, 1.70 (1.08-2.81)	2.61 ± 2.54, 1.92 (0.967-2.83)	2.25 ± 1.23, 1.93 (1.42-2.71)
Total Cholesterol [mg/dL]	150.90 ± 37.79, 145.00 (125.50-173.00)[b]	147.60 ± 27.77, 151.00 (137.50-167.00)[b]	188.30 ± 36.01, 181.00 (160.00-218.00)
LDL-Cholesterol [mg/dL]	84.65 ± 32.71, 81.40 (63.40-106.20)	80.76 ± 26.53, 81.40 (68.20-104.80)[c]	106.80 ± 28.58, 106.00 (87.30-123.80)
HDL-Cholesterol [mg/dL]	37.70 ± 10.95, 37.00 (29.00-44.25)[a]	38.76 ± 9.89, 41.00 (30.50-45.00)[a]	63.12 ± 16.24, 58.00 (49.00-75.50)
Triglycerides [mg/dL]	137.00 ± 62.86, 115.00 (94.00-171.50)[c]	134.60 ± 49.08, 114.00 (98.50-159.00)[c]	91.64 ± 38.62, 79.00 (65.00-119.50)
CRP [mg/dL]	1.13 ± 2.10, 0.30 (0.10-0.90)	1.02 ± 1.61, 0.40 (0.15-1.05)	-
Obesity (n, %)	9, 19.15%	0[b]	9, 36.00%
Central obesity (n, %)	43, 91.00%[a]	15, 88.24%[b]	0
Smoking (n, %)	28, 59.57%[a]	8, 47.06%[a]	0
Hypertension (n, %)	38, 80.85%[a]	14, 82.35%[a]	0
History of CVD (n, %)	20, 42.55%[a]	10, 71.43%[a]	0
Dysplipidemia (n, %)	37, 78.72%[a]	14, 82.35%[a]	0
Diabetes (n, %)	15, 31.91%[a,d]	1, 5.89%	0

Antidiabetic drugs (n, %)	12, 25.53% [b]	1, 5.89%	0
Aspirin (n, %)	36, 76.60% [a]	13, 76.47% [a]	0
ACEI/ARB (n, %)	30, 63.83% [a]	14, 82.35% [a]	0
β-Blockers (n, %)	35, 74.47% [a]	12, 70.58% [a]	0
Ca-channel blokers (n, %)	11, 23.40% [b]	2, 11.76%	0
Statins (n, %)	37, 78.72% [a]	15, 88.24% [a]	0

The table shows the main characteristics of the whole study population (CAD), the subgroup of patients who underwent CABG and the group of healthy subjects. Data are expressed as mean ± SD, median (25th-75th percentiles) or number and proportions. ACEI: angiotensinogen-converting enzyme inhibitor; ARB: angiotensin receptor blockade; BMI, body mass index; CAD, coronary artery disease; CRP, C reactive protein; CVD, cardiovascular diseases; EAT, epicardial adipose tissue; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; WC, waist circumference; WHtR, waist-to-height ratio. HbA1c and CRP data were not available for healthy subjects. a, $p < 0.001$ *vs.* healthy; b, $p < 0.01$ *vs.* healthy; c, $p < 0.05$ *vs*. healthy; d, $p < 0.05$ *vs*. CABG.

Table 2.

Correlation analysis of *GLP1-R* with genes involved in fatty acid metabolism and adipogenesis

a) FATTY ACID METABOLISM GENES

GENES POSITIVELY CORRELATED	FAMILY GROUP	FUNCTION	CORRELATION COEFFICIENT, *p* VALUE
ACAD10	Acyl-CoA Dehydrogenases	Promote FA oxidation	0.543, 0.007
ACADL			0.560, 0.006
ACOT6	Acyl-CoA Thioesterases	Regulate FA oxidation in mitochondria and peroxisomes	0.861, <0.0001
ACOT 12			0.872, <0.0001
ACSBG2	Acyl-CoA Synthetases	Activate long- and medium-chain FA for oxidation	0.915, <0.0001
ACSL6			0.477, 0.021
ACSM3			0.699, 0.0002
ACSM4			0.903, <0.0001
CPT1B	Fatty Acid Transport	Transport FA into mitochondria for oxidation	0.415, 0.049
FABP1	Fatty Acid Transport	Facilitate FA transfer across membranes	0.709, 0.0002
FABP2			0.911, <0.0001
FABP6			0.598, 0.003
FABP7			0.918, <0.0001
GK	Triacylglycerol Metabolism	Esterification of FA with reduced FA efflux	0.664, 0.0006
GK2			0.930, <0.0001
HMGCS2	Ketogenesis & Ketone Body Metabolism	Sintesys and utilization of lipid-derived energy	0.797, <0.0001
OXCT2			0.694, 0.0002
PRKAA1	Fatty Acid Biosynthesis Regulation	Alpha catalytic subunit of AMPK: switch off ATP-consuming biosynthetic pathways	0.612, 0.0019
PRKAA2			0.799, <0.0001
PRKAG2	Fatty Acid Biosynthesis Regulation	Gamma subunit of AMPK: switch off ATP-consuming biosynthetic pathways	0.430, 0.0403
PRKAG3			0.681, 0.0004
SLC27A1	Fatty Acid Metabolism	Long-chain FA import into tissue at high levels of beta-oxidation	0.782, <0.0001

GENES NEGATIVELY CORRELATED	FAMILY GROUP	FUNCTION	CORRELATION COEFFICIENT, *p* VALUE
CROT	Fatty Acid Transport	Transport of medium length acyl chains out of the mammalian peroxisome	-0.522, 0.0106
DECR1	Other Fatty Acid Metabolism Genes	Auxiliary enzyme of beta-oxidation	-0.469, 0.0240
HADHA	Other Fatty Acid Metabolism Genes	Catalyzes the last three steps of mitochondrial beta-oxidation of long chain fatty acids	-0.481, 0.0202
PPA1	Other Fatty Acid Metabolism Genes	Involved in lipid storage	-0.486, 0.0186
PRKAB1	Fatty Acid Biosynthesis	Unit of AMPK: switch off ATP-consuming biosynthetic pathways and pro	-0.414, 0.0498

	Regulation		
EHHADH	Acyl-CoA Dehydrogenases	One of the four enzymes of the peroxisomal beta-oxidation	-0.448, 0.0319

b) ADIPOGENESIS GENES

GENES POSITIVELY CORRELATED	FAMILY GROUP	FUNCTION	CORRELATION COEFFICIENT, *p* VALUE
FOXC2	Pro-Brown	Increase the amount of brown adipose tissue leading to lower weight and an increased sensitivity to insulin	0.815, <0.0001
GATA3			0.736, <0.0001
PPARGC1A			0.574, 0.0420
SRC			0.535, 0.0086
UCP1			0.633, 0.0012
NR0B2	Anti-Brown	Increase the amount of brown adipose tissue and insulin sensitivity	0.838, <0.0001
WNT10B			0.800, <0.0001
CDKN1B	Anti-Adipogenesis	Repress adipogenesis	0.441, 0.0350
DLK1			0.772, <0.0001
LRP5			0.506, 0.0137
WNT1			0.734, <0.0001
WNT3A			0.920, <0.0001
DKK1	Pro-Adipogenesis	Inhibitors of WNT anti-adipogenesis pathway	0.451, 0.0309
SFRP5			0.523, 0.0104
WNT5B			0.454, 0.0297
ADIG	Adipokines	Plays a role in stimulating adipocyte differentiation and development (both brown and white)	0.9239, <0.0001
RETN	Adipokines	Seems to suppress insulin ability to stimulate glucose uptake into adipose cells	0.6822, 0.0003

GENES NEGATIVELY CORRELATED	FAMILY GROUP	FUNCTION	CORRELATION COEFFICIENT, *p* VALUE
MAPK14	Pro-Brown	Increase the amount of brown adipose tissue and insulin sensitivity	-0.475, 0.0220
NRF1			-0.6477, 0.0008
VDR	Anti-Adipogenesis	Repress adipogenesis	-0.566, 0.0048
AXIN1	Pro-Adipogenesis	Stimulate adipocyte differentiation and development	-0.463, 0.0263
CDK4			-0.519, 0.0112
CEBPB			-0,376, 0.0770
LMNA			-0.567, 0.0048
RB1			-0.643, 0.0009
EGR2	Pro-White	Increase the amount of white adipose tissue	-0.643, 0.0009

Table reports existing positive and negative correlations of GLP1-R with genes involved in fatty acid metabolism (a) adipogenesis (b). Spearman correletion coefficients and corresponding *p* values are reported.

Table 3.

Correlation analysis of *GLP2-R* with genes involved in fatty acid metabolism and adipogenesis

a) FATTY ACID METABOLISM GENES

GENES NEGATIVELY CORRELATED	FAMILY GROUP	FUNCTION	CORRELATION COEFFICIENT, *p* VALUE
ACSL6	Acyl-CoA Synthetases	Activation of long-chain fatty acids for degradation via beta-oxidation	-0.419, 0.0466
CPT1A	Carnitine Transferases	FA transport for beta-oxidation	-0.470, 0.0235

b) ADIPOGENESIS GENES

GENES POSITIVELY CORRELATED	FAMILY GROUP	FUNCTION	CORRELATION COEFFICIENT, *p* VALUE
AGT	Hormones	Promote lipid accumulation	0.534, 0.0087
CEBPB	Pro-Adipogenesis	Regulation of genes involved in immune and inflammatory responses	0.5167, 0.0116
NCOR2	Anti-Adipogenesis	Inhibits adipogenic differentiation	0,478, 0.0210
SREBF1	PPARgamma target	Lipid synthesis	0.477, 0.0213

GENES NEGATIVELY CORRELATED	FAMILY GROUP	FUNCTION	CORRELATION COEFFICIENT, *p* VALUE
WNT1	Anti-Adipogenesis	Repress adipogenesis	-0.420, 0.046

Table reports existing positive and negative correlations of *GLP2-R* with genes involved in fatty acid metabolism (a) adipogenesis (b). Spearman correletion coefficients and corresponding *p* values are reported.