

Study of the Potential Association of Adipose Tissue GLP-1 Receptor with Obesity and Insulin Resistance

Joan Vendrell, Rajaa El Bekay, Belén Peral, Eduardo García-Fuentes, Anna Megia, Manuel Macias-Gonzalez, José Fernández Real, Yolanda Jimenez-Gomez, Xavier Escoté, Gisela Pachón, Rafael Simó, David M. Selva, María M. Malagón, and Francisco J. Tinahones

University Hospital of Tarragona Joan XXIII (J.V., A.M., X.E., G.P.), IISPV, Rovira i Virgili University, Centro de Investigación Biomédica en Red Diabetes y Enfermedades Metabólicas Asociadas, Instituto de Salud Carlos III, 43007 Tarragona, Spain; Centro de Investigación Biomédica en Red Fisiopatología Obesidad y Nutrición (CB06/03) (R.E.B., E.G.-F., M.G., Y.J.-G., F.J.T.), Instituto de Salud Carlos III, 29010 Málaga, Spain; Laboratorio de Investigación Biomédica (R.E.B., E.G.-F., M.G., Y.J.-G., F.J.T.), Hospital Clínico Universitario Virgen de la Victoria, and Servicio de Endocrinología (F.J.T.), Hospital Virgen de la Victoria, 29010 Málaga, Spain; Instituto de Investigaciones Biomédicas (B.P.), Alberto Sols, Consejo Superior de Investigaciones Científicas and Universidad Autónoma de Madrid, E-28029 Madrid, Spain; Service of Diabetes, Endocrinology, and Nutrition (J.F.R.), Institut d'Investigacion Biomedica de Girona, Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CB06/03/0010) and Instituto de Salud Carlos III, 17007 Girona, Spain; Department of Cell Biology, Physiology, and Immunology (Y.J.-G., M.M.M.), Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CB06/03/0020) and Instituto de Salud Carlos III, Instituto de Investigación Biomédica, University of Córdoba, 14004 Córdoba, Spain; Centro de Investigación Biomédica en Red Diabetes y Enfermedades Metabólicas Asociadas and Institut de Recerca Hospital Universitari Vall d'Hebron (R.S., D.M.S.), 08035 Barcelona, Spain

The increase in glucagon-like peptide-1 (GLP-1) activity has emerged as a useful therapeutic tool for the treatment of type 2 diabetes mellitus. The actions of GLP-1 on β -cells and the nervous and digestive systems are well known. The action of this peptide in adipose tissue (AT), however, is still poorly defined. Furthermore, no relationship has been established between GLP-1 receptor (GLP-1R) in AT and obesity and insulin resistance (IR). We provide evidence for the presence of this receptor in AT and show that its mRNA and protein expressions are increased in visceral adipose depots from morbidly obese patients with a high degree of IR. Experiments with the 3T3-L1 cell line showed the lipolytic and lipogenic dose-dependent effect of GLP-1. Moreover, GLP-1 stimulated lipolysis in 3T3-L1 adipocytes in a receptor-dependent manner involving downstream adenylate cyclase/cAMP signaling. Our data also demonstrate that the expression of the GLP-1R in AT correlated positively with the homeostasis model assessment index in obese IR subjects. Furthermore, prospective studies carried out with patients that underwent biliopancreatic diversion surgery showed that subjects with high levels of GLP-1R expression in AT, which indicates a deficit of GLP-1 in this tissue, were those whose insulin sensitivity improved after surgery, suggesting the potential relationship between AT GLP-1R and insulin sensitivity amelioration in obese subjects. Altogether these results indicate that the GLP-1/GLP-1R system in AT represents another potential candidate for improving insulin sensitivity in obese patients. (*Endocrinology* 152: 4072–4079, 2011)

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Abbreviations: AT, Adipose tissue; BPD, biliopancreatic diversion technique; Ct, cycle number at which the detected fluorescence exceeds the threshold; DM2, type 2 diabetes mellitus; GIP, glucose-dependent insulintropic polypeptide; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; HOMA, homeostasis model assessment; IBMX, isobutylmethylxanthine; IR, insulin resistance; MDL-12,330A, [cis-*N*-(2-phenylcyclopentyl) azacyclotridec-1-en-2-amine HCl]; OB/H-IR, morbidly obese patients with high insulin resistance degree; OB/L-IR, morbidly obese patients with low insulin resistance degree; SAT, sc AT; VAT, visceral AT.

The prevalence of obesity is increasing and has reached epidemic proportions. Obesity is frequently associated with the inability of tissue to handle insulin, with a clear tendency to develop an insulin-resistant environmental factor. The accepted events in the context of insulin resistance (IR) include increased insulin production, leading to hyperinsulinemia, and the compensatory failure of insulin secretion by the β -cells, which leads to the emergence of type 2 diabetes mellitus (DM2) (1). In this scenario, visceral fat plays a determinant role linking central obesity with the onset of IR (2–4). Furthermore, other data suggest the link between adipose cell size, increased insulin resistance, and diabetes (5).

Epidemiological studies have established a strong correlation between obesity and diabetes, with population-based studies showing a linear increase in the rate of diabetes for every kilogram gained (6).

However, multiple consistent lines of evidence indicate that no clear cutoff for developing IR can be defined for a specific degree of obesity due to the paradox that some morbidly obese subjects do not develop diabetes, whereas some lean subjects do. Thus, several studies have focused on the analysis of adipose tissue dysfunction as one of the driving factors in the development of insulin resistance and finally DM2.

Glucagon-like peptide 1 (GLP-1) amide (7–36) is an enteroendocrine-derived peptide secreted in response to nutrient ingestion. GLP-1 stimulates insulin biosynthesis and secretion in a glucose-dependent manner, thus increasing the sensitivity to glucose (7, 8). In addition, GLP-1 improves α -cell glucose sensing in patients with DM2 (9). GLP-1 also inhibits gastric emptying and controls food intake by increasing satiety in treated DM2 patients (10, 11).

The effect of GLP-1 in adipose tissue (AT) has been poorly studied, and only a few reports exist on this issue. Studies performed in isolated rat and human adipocytes (12–15) have demonstrated that GLP-1 has the ability to induce both lipogenic and lipolytic mechanisms, with *in vivo* studies showing inhibition of fasting lipolysis (16). These GLP-1 effects in fat, like those in liver and muscle, are exerted through a GLP-1-specific receptor and structurally or functionally distinct from that expressed in the pancreas (17). Furthermore, the presence of GLP-1 receptor (GLP-1R) and its function have been widely studied in 3T3-L1 adipocytes (18), whereas the molecular identity of GLP-1R in human AT remains unclear.

The main objective of this work was to verify the presence of GLP-1R in human AT and correlate the expression levels of this receptor with the degree of insulin resistance.

Materials and Methods

Subjects and processing of biological samples

This section is detailed in Supplemental Data 1, published on The Endocrine Society's Journals Online web site at <http://endo.endojournals.org>. Anthropometric measurements are shown in Supplemental Data 2. Analytical methods are shown in Supplemental Data 2.

Western blotting analysis

Proteins were extracted from samples [sc AT (SAT) and visceral AT (VAT)], pulverized in liquid nitrogen, homogenized, and lysed in 1:20 (wt/vol) of T-PER reagent and protease inhibitor cocktail, sonicated, and centrifuged. The protein concentration of each supernatant was quantified using a Coomassie protein assay kit. About 20 μ g of protein was separated into 15% sodium dodecyl sulfate-polyacrylamide gel and transferred to polyvinylidene difluoride membranes. These membranes were incubated overnight at 4 C with antibodies to GLP-1R (Abcam, Cambridge, MA) or monoclonal anti- β -actin, followed by incubation with horseradish peroxidase-conjugated secondary antibody. The proteins were visualized with SuperSignal West Pico chemiluminescent substrate (Thermo Fisher Scientific) and quantified by Auto-Chem system and Labworks 4.6 image acquisition analysis software (UVP, Inc., Upland, CA). Data were normalized to the corresponding β -actin band intensities.

Immunohistochemistry of AT

Five-micron sections of formalin-fixed, paraffin-embedded human AT were deparaffinized and rehydrated before antigen unmasking with pepsin at 37 C. Sections were blocked in normal serum and incubated overnight with mouse antihuman GLP-1R at 1:100 dilutions. Secondary antibody staining was performed using the VECTASTAIN ABC kit (Vector Laboratories, Burlington, Ontario, Canada) and detected with diaminobenzidine. Sections were counterstained with hematoxylin before dehydration and coverslip placement. As a negative control, the entire immunohistochemical procedure was performed on adjacent sections in the absence of primary antibodies (Abcam, Cambridge, MA).

3T3-L1 cell culture and *in vitro* experimental setups

In vitro experiments were performed on 3T3-L1 cells differentiated by adipocytes, as previously described (17). Briefly, cells were grown in DMEM (Cambrex Bio Science, Vervies, Belgium), supplemented with 10% fetal bovine serum (Sera-Lab Ltd., Crawley Down, UK), 4 mM glutamine, and 1,5 g/liter H_2CO_3 (culture medium) at 37 C and 5% CO_2 until 100% confluence was reached (d 0). Subsequently, cells were incubated in DMEM containing 0.5 mM isobutylmethylxanthine (IBMX), 0.25 μ M dexamethasone, and 10 μ g/ml insulin for 72 h (d 3). Thereafter the medium was replaced by DMEM containing 10 μ g/ml insulin for an additional 72 h period (d 6) and then exchanged by culture medium until d 10, when all the experiments were carried out. On the day of the experiments, differentiated 3T3-L1 adipocytes were preincubated in 1 ml serum-free culture medium (DMEM, 1 g/liter glucose) for 2 h. Medium was then replaced by DMEM alone or containing 10 nM or 100 nM GLP-1 (fragment 7–36, Sigma G8147; Sigma-Aldrich, London, UK). After a 12-h treat-

ment period, cells were harvested for RNA analysis and protein quantification, and supernatants were used to determine lipolysis. In another set of experiments, we explored the contribution of the adenylate cyclase/cAMP pathway to GLP-1 lipolytic response by incubating cells for 4 h with 100 nM GLP-1 in the presence or absence of the specific blocker of the enzyme, [cis-N-(2-phenylcyclopentyl) azacyclotridec-1-en-2-amine HCl] (MDL-12,330A; 10^{-6} M). This inhibitor was added alone to cell cultures 90 min before the 4-h combined treatment. Medium samples were collected at the end of each experiment.

Measurement of cAMP production

To elucidate the effect of GLP-1 on intracellular cAMP production, differentiated 3T3-L1 cells were incubated for 60 min in the absence or presence of GLP-1 (100 nM) or the GLP-1 receptor antagonist exendin (9–39) (10 nM) alone or in combination. The effects of glucagon (10 nM) and glucose-dependent insulinotropic polypeptide (GIP)-1 (10 nM) on intracellular cAMP accumulation in 3T3-L1 cell cultures were also assessed. At the end of the experiments, media were removed, lysis buffer was added, and lysates were removed and stored at -20 C for analysis of intracellular cAMP accumulation by enzyme immunoassay according to the manufacturer's instructions (cAMP Direct Bio-track enzyme immunoassay; GE Healthcare, Barcelona, Spain).

Unless otherwise indicated, all other reagents were purchased from Sigma Aldrich.

Human preadipocyte isolation and primary culture

Abdominal white adipose tissue samples were washed twice with PBS and minced removing the blood vessels. Samples were then digested in a water bath with collagenase I ($1 \mu\text{g/ml}$) at 37 C for 30 min followed by several washes in PBS, filtrations, and centrifugations (800 rpm) with 250 and 100 μm nylon meshes. Erythrocytes were removed with red blood cell lysis buffer (155 mM NH_4Cl , 10 mM KHCO_3 , 2 mM NaEDTA), and cells were centrifuged for 5 min at 800 rpm. Preadipocytes were then re-suspended in **human omental preadipocyte medium** [DMEM/Hams F-12 medium (1:1, vol/vol); HEPES pH 7.4; fetal bovine serum; penicillin; streptomycin; amphotericin B] and plated in 12- and 96-well plates. Media were changed every 2–3 d until cells reached confluence. For the differentiation protocol, cells were cultured with OM-DM [DMEM/Hams F-12 medium (1:1, vol/vol); HEPES pH 7.4; fetal bovine serum; biotin; pantothenate; human insulin; dexamethasone; IBMX; peroxisomal proliferator-activated receptor- γ agonist; penicillin; streptomycin; amphotericin B] for 1 wk changing the media every 2–3 d, followed by another week in **human omental adipocyte medium** [DMEM/Hams F-12 medium (1:1, v/v); HEPES, pH 7.4; fetal bovine serum; biotin; pantothenate; human insulin; dexamethasone; penicillin; streptomycin; amphotericin B]. Mature adipocytes were then treated with different concentrations of GLP-1 (10 nM, 100 nM, and 1 μM) for 12 h before cells were harvested for RNA isolation.

Lipolysis assays

After these experimental treatments, media from 3T3-L1 and human primary cell cultures were collected and the samples were analyzed for free glycerol content using free glycerol reagent (Sigma-Aldrich) following the manufacturer's indications. Glycerol content was normalized to protein concentrations in each sample.

Total RNA isolation and real-time PCR

Total RNA was extracted from 3T3-L1, human preadipocyte cell cultures, and human AT samples using TRIZOL reagent. Total RNA samples were diluted to concentrations between 100 and 200 ng/ μl . An aliquot of the diluted total RNA samples was used to determine RNA concentration and purity on the Nano-Drop ND-1000 spectral photometer (Peqlab, Wilmington, DE). Subsequently, mRNA amplifications were carried out using a MicroAmp optical 96-well reaction plate (PE Applied Biosystems, Foster City, CA) on an ABI 7500 real-time PCR system. The amplifications were performed with MicroAmp optical 96-well reaction plate (PE Applied Biosystems) on an ABI 7500 real-time PCR system (Applied Biosystems). Quantitative RT-PCR reactions were carried out for all genes using specific TaqMan gene expression assays. A negative PCR control without template and a positive PCR control with a template of known amplification were included in each assay. During PCR the Ct values (the cycle number at which the detected fluorescence exceeds the threshold) for each amplified product were determined using a threshold value of 0.1. The specific signals were normalized using a threshold value of 0.1. The specific signals were normalized by constitutively expressed cyclophilin and 18s rRNA (as indicated in each figure) signals using the formula $2^{-\Delta\text{Ct}}$ for mRNA expression in tissue fractions extracted from human AT and $2^{-\Delta\Delta\text{Ct}}$ for cell cultures incubated in the presence of GLP-1 taking the untreated control as a calibrator.

Statistical analyses

SPSS statistical software, version 11.0 for Windows (SPSS Inc., Chicago, IL) was used for statistical analyses. The normal distribution of variables to characterize differences in gene expression or lipolytic rates was assessed using the Kolmogorov-Smirnov test, followed by the one-way ANOVA test for the comparison of the different groups, and the statistical differences were carried out by the use of Duncan's test. *Post hoc* statistical analyses were completed by using the protected least-significant-difference test to identify significant differences between treatments *in vitro*. The Student *t* test was used for the

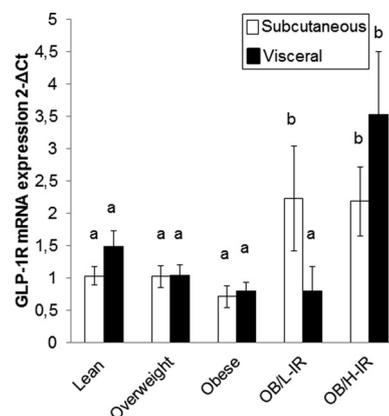


FIG. 1. GLP-1R mRNA expression in SAT and VAT. GLP-1R mRNA expression analysis was performed in human SAT and VAT from lean, overweight, obese, and morbidly obese subjects with low (OBL-IR) and high (OBLH-IR) degrees of insulin resistance. mRNA were normalized to cyclophilin levels. Results were obtained in triplicate and expressed as the mean \pm SD ($n = 18$ for lean, $n = 36$ for overweight, $n = 19$ for obese, and $n = 22$ for morbidly obese subjects). Bars with different letters have a significant difference ($P < 0.05$).

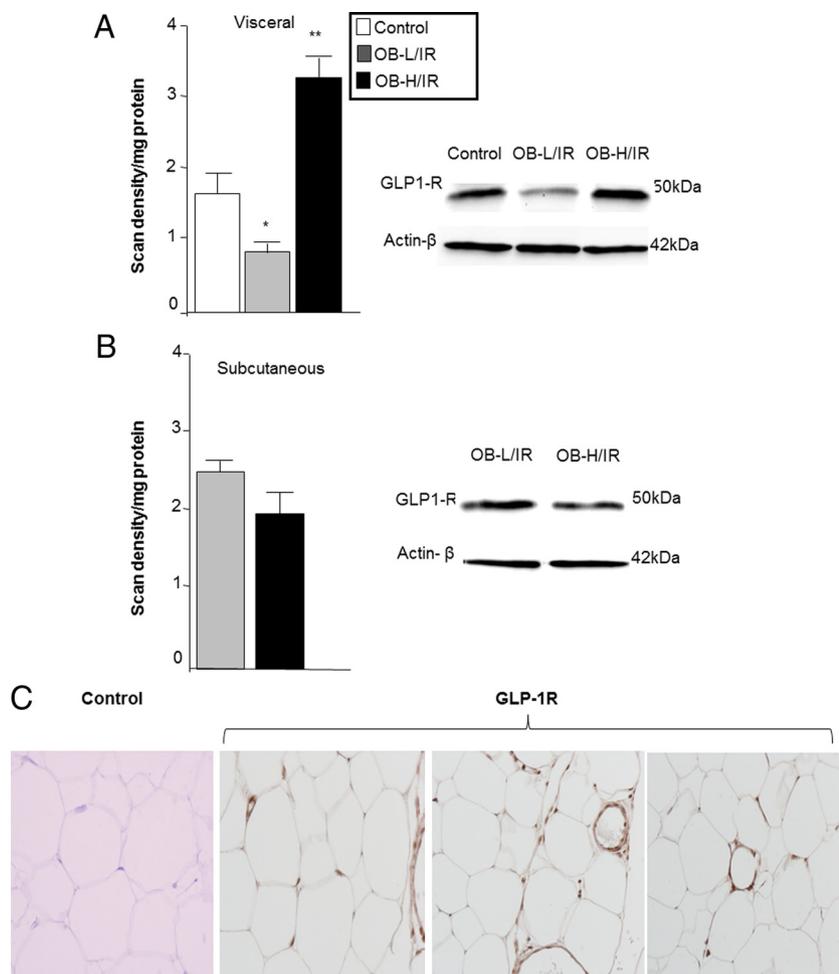


FIG. 2. GLP-1R protein expression in human AT. Western blot analysis of human AT yielded a single 50-kDa band in both visceral (A) and subcutaneous (B) tissue corresponding to the known molecular weight of human GLP-1R. Monoclonal anti- β -actin was applied as a loading control. The blot is representative of three independent experiments with different samples ($n = 6$ lean, $n = 5$ OB/L-IR, and $n = 6$ OB/H-IR). *, Significance in the t test is indicated by $P < 0.05$; **, significance in the t test is indicated by $P < 0.01$. C, Immunohistochemical identification of GLP-1R in human VAT was performed using a specific antibody against this protein. Control panel is representative of *blue labeling* corresponding to hematoxylin-labeled nucleus. Positive cells showed *brown labeling*, especially at the mesothelium, endothelium, and nucleus.

paired data of morbidly obese subjects before and after gastric bypass surgery. Differences were considered significant at $P < 0.05$. All data presented in the text and figures are expressed as mean \pm SD. The correlation analysis of mRNA quantitative expression for each of the genes was performed with Spearman's coefficient test (r). One-way ANOVA followed by a Newman-Keuls test were used for parametric data in cAMP study.

Results

Anthropometric and biochemical characteristics of the study groups

Table 1 (Supplemental Data 3) shows the main clinical and analytical data of the study population grouped by body mass index and functional IR status. Body mass in-

dex and waist circumference increased progressively from overweight to morbidly obese subjects. Glucose levels were similar across the groups. No differences in the homeostasis model assessment (HOMA) index were observed between the overweight, obese, and OB/L-IR groups compared with the lean subjects, as intended in the study design because we selected only those who had a low degree of IR; however, HOMA index was significantly increased in the OB/H-IR group compared with lean subjects. These results were consistent with what we were expecting because only nonmorbidly obese subjects with a low degree of IR were included in the first three groups of the table (Supplemental Data 3).

Adiponectin was significantly lower only in the OB/H-IR group compared with the other groups. Nevertheless, no significant differences were detected between OB/L-IR, obese, overweight, and lean subjects.

GLP-1R gene and protein expression in AT

When we analyzed GLP-1R gene expression in both adipose depots, we observed that there was a significant progressive decrease in the VAT depot among the different obesity groups (from lean to OB/L-IR morbidly obese subjects). In contrast to the insulin-sensitive lean, overweight, and obese groups and the low insulin-resistant group, the GLP-1R mRNA expression was increased by approximately 2.5-fold in the high insulin-resistant obese group (Fig. 1).

In SAT, no differences in GLP-1R gene expression were observed between lean, overweight, and obese subjects. However, a substantial increase in GLP-1R gene expression was observed in both groups of morbidly obese subjects compared with the lean group.

Regarding GLP-1R protein expression, we observed a significantly reduced expression in VAT depots from OB/L-IR subjects when compared with the lean group. Conversely, for OB/H-IR subjects, GLP-1R protein expression was found to be significantly higher compared with both OB/L-IR and lean subjects (Fig. 2A). No differences were observed in SAT depots (Fig. 2B).

To evaluate the weight of the different components of the AT contributing to GLP-1R expression, immunostain-

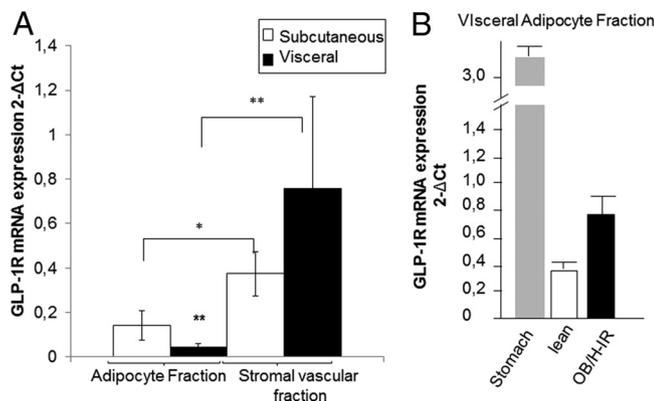


FIG. 3. GLP-1R mRNA expression in adipocyte and stromal-vascular fractions from human adipose tissue. A, Adipocyte and stromal-vascular fractions were isolated from SAT and VAT, and then GLP-1 receptor mRNA expression was analyzed in both. B, GLP-1R mRNA in VAT adipocyte fraction was compared with that in stomach samples, which represents a positive control of GLP-1R gene expression. Results were obtained from triplicate experiments and expressed as the mean \pm SD. *, $P < 0.05$; **, $P < 0.001$.

ing using a specific GLP-1R antibody was assayed. Both the adipocyte and stromal-vascular fraction showed a positive reaction, with a greater intensity in the latter (Fig. 2C).

GLP-1R gene expression in stromal-vascular and adipocyte fraction from VAT and sc human adipose tissue

Real-time PCR analysis of GLP-1R gene expression in both stromal vascular and adipocyte fractions confirmed the previous results obtained by immunostaining described above. Thus, in a pool sample obtained from seven lean and obese subjects, GLP-1R mRNA was expressed both in adipocyte and stromal vascular fractions (Fig. 3A), not at levels usually observed in stomach samples, in which this receptor is known to be highly expressed but still significantly high (Fig. 3B). A clear increase (almost 2.6-fold) was observed in stromal vascular fraction compared with isolated adipocytes mainly in VAT depots (Fig. 3A).

Effects of GLP-1 on lipolysis and cAMP production

To assess the effect of GLP-1 on adipocyte function, we incubated differentiated adipocytes from the 3T3-L1 mouse cell line, in the absence or presence of 10 and 100 nM GLP-1. The addition of GLP-1 to culture cells induced a significant enhancement of lipolysis measured by glycerol release into the media (Fig. 4A). We subsequently determined whether a major intracellular effector activated by GLP-1, adenylate cyclase, could be involved in the lipolytic response of 3T3-L1 adipocytes to the peptide. As shown in Fig. 4B, the specific adenylate cyclase inhibitor MDL-12,330A (10^{-6} M), which by itself did not modify basal lipolysis, suppressed the stimulation caused by GLP-1 on the lipolytic activity of 3T3-L1 cells. On the

basis of these results, our next experiments attempted to examine the effect of GLP-1 on cAMP production. Incubation of 3T3-L1 cells with 100 nM GLP-1 increased cAMP content almost 3-fold with respect to that observed in controls (Fig. 4C). The GLP-1-induced increase in cAMP levels was blocked when the peptide was administered in the presence of the GLP-1 specific receptor blocker exendin (9–39) (Fig. 4C). The effects of glucagon and GIP, both of which are known to elevate cAMP levels, were also assessed in 3T3-L1. This showed that cAMP levels induced by GLP-1 were slightly lower than those induced by GIP and glucagon (Fig. 4C).

To further confirm the above results, we replicated the study in human mature adipocytes differentiated from AT-derived mesenchymal stem cells. GLP-1 treatment in differentiated mature adipocytes induced a significant lipolytic effect in a dose-dependent manner, reaching 50% of the levels observed in the presence of the positive control obtained after incubating the cells with IBMX (Fig. 4D).

Prospective study in the morbidly obese cohort

This cohort was scheduled for bariatric surgery and was reevaluated at the 6-month follow-up. In Table 2 of the Supplemental Data 3, we represent the main anthropometrical and metabolic variables of the morbidly obese cohort, taken before surgery and 6 months later. As was expected after massive weight loss, lipid and metabolic parameters were notably improved in this cohort, with a dramatic decrease in the HOMA index in the whole population. Likewise, plasma adiponectin was increased with an expected decrease in leptin circulating levels at the end of the follow-up (Table 2 of the Supplemental Data 3).

Regarding GLP-1R adipose tissue expression, we observed a strong positive correlation between GLP-1R gene expression in VAT depots with the HOMA index and waist circumference ($r = 0.485$, $P = 0.19$ and $r = 0.351$, $P = 0.002$, respectively) (Fig. 5, A and B).

Interestingly, when we sought variables determining improvement in the HOMA index after bariatric surgery, we found that GLP-1R expression levels in VAT depots correlated positively with the decrease in the HOMA index (Fig. 5, C and D).

Discussion

In the present work, we have shown for the first time that GLP-1R in adipose tissue is potentially associated with the degree of insulin resistance. Thus, we observed that subjects with morbid obesity and a high degree of IR display a clear increase in GLP-1R gene and protein expression in visceral adipose tissue. In addition, GLP-1R mRNA ex-

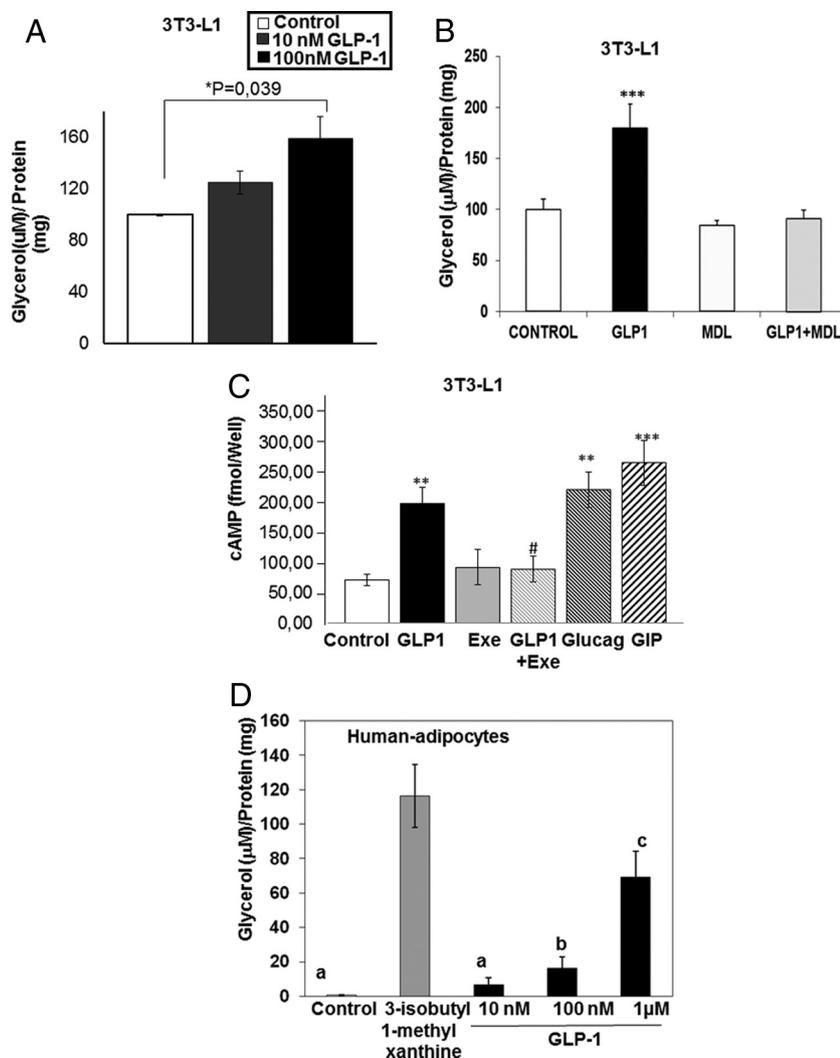


FIG. 4. Effect of GLP-1 on lipolysis in human adipocytes and 3T3-L1 cells. A–C, Mouse embryonic 3T3-L1 cells ($n = 6$) were differentiated into adipocytes and then treated with 10 or 100 nM GLP-1 for 12 h (A) or with 100 nM GLP-1 for 4 h in the presence or absence of the adenylate cyclase inhibitor MDL 12,330 A (10^{-6} M). Cells were harvested to determine lipolysis (B). ***, $P < 0.01$ vs. control. C, For cAMP measurements, 3T3-L1 cells were incubated for 1 h with GLP-1 (100 nM) or exendin (9–39) (Exe; 10 nM), alone or in combination, and in the presence of glucagon (Glucag; 10 nM) or GIP (10 nM) ($n = 4$). Thereafter cAMP production was measured. **, $P < 0.01$ vs. control; ***, $P < 0.001$ vs. control; #, $P < 0.05$ vs. GLP-1 alone. D, Human preadipocytes were isolated from VAT stromal-vascular fraction, differentiated into mature adipocytes, and then treated with different concentrations of GLP-1 (10 nM, 100 nM, and 1 μM) for 12 h. Glycerol release was evaluated in mature adipocytes treated with GLP-1 and 3-isobutyl-1-methylxanthine. Bars with different letters have a significant difference ($P < 0.05$).

pression in VAT depots was elicited as an important determinant of the HOMA index before and after surgical weight loss in morbidly obese patients.

Until now, few studies have addressed GLP-1R in human AT; in fact, when listing the tissues wherein GLP-1 exerts its action, AT is usually overlooked (7, 8, 10, 11). Hence, there are no published data relating to human adipose tissue GLP-1R expression dependent on the degree of insulin resistance. The presence of the GLP-1 receptor in isolated human and mouse adipocytes has been re-

ported since the 1990s (18–20). In the present work, we further extended these findings by demonstrating the expression of GLP-1R, both at the mRNA and protein levels, in the two main components of AT, mature adipocytes and stromal-vascular cells, with a clear predominance in the latter. Furthermore, immunohistochemical analysis enabled us to unveil the presence of GLP-1R in the mesothelium, stromal-vascular fraction, and adipocytes.

The picture displayed by GLP-1R expression in the study cohort showed a different behavior, depending on the adipose tissue depot analyzed. In SAT, no differences in GLP-1R expression were noted in obese subjects with low degrees of IR. In extremely obese patients classified as morbidly obese (OB), a different pattern was observed in our study. In these patients, in SAT depots, we observed a substantial increase of GLP-1R expression when compared with the nonmorbidly obese cohort. Interestingly, functional classification of OB subjects according IR status revealed that GLP-1R in VAT depots was dramatically up-regulated in the setting of a very high degree of insulin resistance. Morbidly obese patients are at the top of the scale, representing the maximum expression of continuous AT growth. This subset of patients carries the highest morbidity risk because it is assumed that many cardiovascular risk factors, including extreme IR, negatively influence their life expectancy (21). However, AT mass by itself is not sufficient to link these patients with a poorer metabolic profile.

In view of the differences observed in GLP-1R expression in VAT depots from OB-IR patients, one is tempted to speculate about the possible compensatory mechanisms needed to overcome proportionally lower GLP-1 levels, when extreme IR is present. In this line, several reports have demonstrated that insulin resistance and diabetes present a clear decrease in circulating GLP-1 after glucose overload (22). The expression of GLP-1R in SAT depots showed no differences between patients with high and low degrees of IR. It is worth mentioning that visceral fat is the

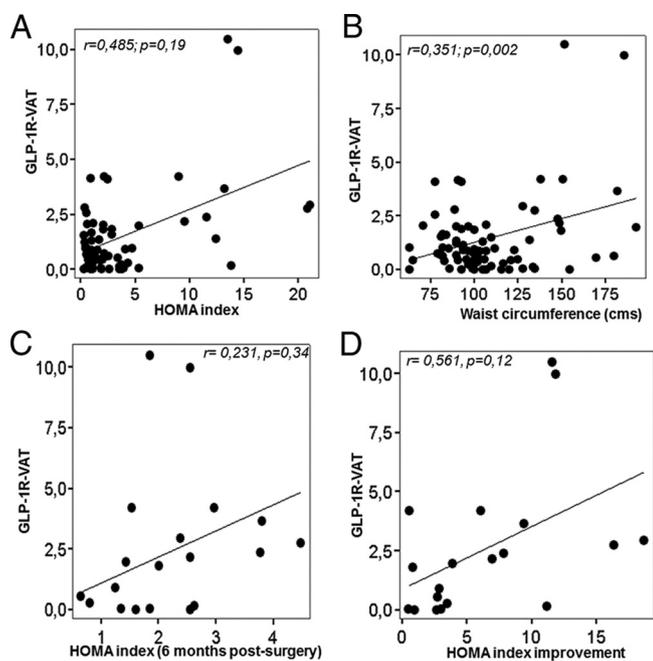


FIG. 5. Correlative analysis of GLP-1R mRNA expression in VAT with HOMA index before and 6 months after surgery and with waist circumference. HOMA index was evaluated in morbidly obese subjects before (A) and 6 months after surgery (C). GLP-1R mRNA expression in VAT correlation with waist circumference (B) and with HOMA index improvement 6 months after surgery (D) were also analyzed. The correlations were determined by Pearson's correlation coefficient test (r).

main component linked to insulin resistance, and there are many reports that describe the close association between this fat depot and several components of the metabolic syndrome. In fact, IR may be the consequence of expanded adipose visceral fat, and in turn, visceral fat may have a different genetic profile, induced by an insulin resistant state. We think that the GLP-1R expression differences observed in VAT depots may generate in part a high IR milieu, at least in the morbidly obese. The association observed between GLP-1R expression in VAT before surgery and the HOMA index improvement after 6 months of massive weight loss reinforce the determinant role of the GLP-1R gene expression in the IR state.

It is worth noting that bariatric surgery improves insulin sensitivity beyond the weight decline. At initial stages after surgery, IR improves without a significant decline in weight (23). This initial insulin sensitivity improvement could be due to the changes in incretin secretion, especially GLP-1. We believe that it is reasonable to propose that those subjects with increased adipose tissue GLP-1R levels and with a deficit of circulating GLP-1 are those who could improve their degree of insulin resistance after surgery.

Several studies have suggested that sustained treatment with GLP-1R agonists is associated with improvements in insulin sensitivity (24–26). Furthermore, it is well estab-

lished that GLP-1 enhancement improves insulin sensitivity in peripheral tissues (16), including muscle, liver, and pancreas. However, the mechanisms by which the GLP-1R in AT could participate in this improvement remain to be defined. We believe that the findings reported herein support the relationship between this peptide and insulin sensitivity. Moreover, GLP-1 is considered to be an important gastrointestinal peptide that plays an active role in lipolysis and fatty acid synthesis, and in 3T3-L1 adipocytes it mediates increased insulin-dependent glucose uptake by up-regulation of some insulin signaling molecules, such as phosphorylated insulin receptor substrate-1, Akt, and glycogen synthase kinase 3b (12, 27). These data, together with ours, could explain the important correlation between insulin resistance and expression levels of GLP-1R in VAT and also the relation of this receptor with insulin sensitivity changes observed after bariatric surgery.

On the other hand, it is known that GLP-1 modulates the expression of the facilitative glucose transporters, glucose transporter-1 and glucose transporter-4, in the 3T3-L1 cell line (28). Here GLP-1 treatment of fully differentiated 3T3-L1 cells induced a dose-dependent lipolytic effect in these cells. In this sense, it is well known that GLP-1 may exert a dose dependent dual action, with predominant lipogenic activity when using picomolar concentrations or lipolytic activity when using nanomolar doses (15). This is in agreement with the observed dose-dependent lipolytic effect observed in both mouse and human mature differentiated adipocytes. Moreover, our data strongly support the view that GLP-1 stimulates lipolysis in adipocytes in a receptor-dependent manner involving downstream adenylate cyclase/cAMP signaling. This hormesis-like response may be behind the different effects on weight loss observed in patients when treated with GLP-1 analogs. The former are characterized by re-establishing GLP-1 physiological levels, whereas analogs of GLP-1 reach supraphysiological doses (as drugs) with an anorexigenic and lipolytic effect leading to weight loss. We are aware that this hypothesis may sound quite speculative, but it may prove attractive to understand these differences better.

Finally, we believe that a better understanding of how the GLP-1 receptor in VAT improves insulin sensitivity in obese subjects would provide additional insight into the pathogenesis of IR.

Acknowledgments

Address all correspondence and requests for reprints to: Dr. Rajaa El Bekay, Laboratorio de Investigación Biomédica, Hospital

Clínico Universitario Virgen de la Victoria, Campus de Teatinos s/n, Málaga 29010 Spain. E-mail: elbekay@gmail.com.

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