Disruption of GIP/GIPR Axis in Human Adipose Tissue Is Linked to Obesity and Insulin Resistance

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Context: Glucose-dependent insulinotropic peptide (GIP) has a central role in glucose homeostasis through its amplification of insulin secretion; however, its physiological role in adipose tissue is unclear.

Objective: Our objective was to define the function of GIP in human adipose tissue in relation to obesity and insulin resistance.

Design: GIP receptor (GIPR) expression was analyzed in human sc adipose tissue (SAT) and visceral adipose (VAT) from lean and obese subjects in 3 independent cohorts. GIPR expression was associated with anthropometric and biochemical variables. GIP responsiveness on insulin sensitivity was analyzed in human adipocyte cell lines in normoxic and hypoxic environments as well as in adipose-derived stem cells obtained from lean and obese patients.

Results: GIPR expression was downregulated in SAT from obese patients and correlated negatively with body mass index, waist circumference, systolic blood pressure, and glucose and triglyceride levels. Furthermore, homeostasis model assessment of insulin resistance, glucose, and G protein-coupled receptor kinase 2 (GRK2) emerged as variables strongly associated with GIPR expression in SAT. Glucose uptake studies and insulin signaling in human adipocytes revealed GIP as an insulin-sensitizer incretin. Immunoprecipitation experiments suggested that GIP promotes the interaction of GRK2 with GIPR and decreases the association of GRK2 to insulin receptor substrate 1. These effects of GIP observed under normoxia were lost in human fat cells cultured in hypoxia. In support of this, GIP increased insulin sensitivity in human adipose-derived stem cells from lean patients. GIP also induced GIPR expression, which was concomitant with a downregulation of the incretin-degrading enzyme dipeptidyl peptidase 4. None of the physiological effects of GIP were detected in human fat cells obtained from an obese environment with reduced levels of GIPR.

Conclusions: GIP/GIPR signaling is disrupted in insulin-resistant states, such as obesity, and normalizing this function might represent a potential therapy in the treatment of obesity-associated metabolic disorders. ***(J Clin Endocrinol Metab* 99: E908–E919, 2014)**

Incretin hormones, released from the gastrointestinal tract, have emerged as important participants in glucose homeostasis by acting on different targets involved in the pathogenesis of type 2 diabetes (T2D). Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) incretins stimulate glucose-dependent insulin biosynthesis and secretion in pancreatic β cells after food ingestion to coordinate energy assimilation (1).

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Abbreviations: BMI, body mass index; DPP4, dipeptidyl peptidase 4; GIP, glucose-dependent insulinotropic peptide; GIPR, GIP receptor; GLP-1; glucagon-like peptide 1; GPCR, G protein-coupled receptor; GRK2, GPCR kinase 2; hADSCs, human adipose-derived stem cells; HOMA-IR, homeostasis model assessment of insulin resistance; IRS1, insulin receptor substrate 1; LPL, lipoprotein lipase; SAT, sc adipose tissue; SBP, systolic blood pressure; SVF, stromal vascular fraction; T2D, type 2 diabetes; VAT, visceral adipose tissue.

Long-acting GLP-1 analogs (incretin mimetics) and highly selective inhibitors of the incretin-degrading enzyme dipeptidyl peptidase 4 (DPP4) are widely accepted treatments to improve glycemic control in the management of T2D (2). Unlike GLP-1, GIP is not considered a useful therapeutic agent for T2D because of the nonresponsiveness of β-cells to GIP in hyperglycemia (3). Emerging data, however, suggest important extrapancreatic metabolic functions for this incretin. Genome-wide association studies recently revealed the GIP receptor (GIPR) as a new locus associated with body mass index (BMI) (4). Also, results from mainly animal studies point to GIPR antagonism, or reducing circulating GIP levels, as beneficial treatments for obesity (5–8). However, transgenic overexpression of GIP, in the context of overnutrition, has also shown major beneficial effects on both glucose and fat metabolism (9).

Recent research has highlighted the relevance of the GIP/GIPR axis in principal insulin-sensitive organs such as adipose tissue. In this setting, GIP has been shown to augment insulin-induced lipogenesis, inhibit the lipolytic action of glucagon, and stimulate lipoprotein lipase (LPL) activity (10, 11). However, the metabolic effects of GIP on adipose tissue have been examined primarily in murine cells, and there is debate concerning the effect of GIP on glucose metabolism. Hence, depending on the experimental conditions, GIP has been described either as an insulin mimetic (12) or an insulin sensitizer (13–15). Other studies point to GIP as an inflammatory and lipolytic factor inducing insulin resistance (16, 17). Interestingly, analysis of GIPR mRNA expression in the adipose tissue of nondiabetic postmenopausal women reveals a decreased expression of the receptor, which is associated with signs of insulin resistance (18).

Here, we explore the GIP/GIPR signaling axis in adipose tissue in the setting of obesity and associated insulin resistance. We demonstrate in 3 different cohorts that GIPR expression is inversely associated with insulin resistance in sc adipose tissue (SAT) from obese patients. Moreover, insulin sensitivity assessed by homeostasis model assessment of insulin resistance (HOMA-IR) emerges as a strongly associated variable, determining GIPR expression in SAT. Studies with human adipocyte cell lines, and also adipocytes differentiated from human adipose-derived stem cells (hADSCs), point to GIP as an insulin-sensitizer incretin. Significantly, we found that adipocytes from an obese environment display a GIP-resistant phenotype. Thus, rescue of the impaired incretin actions on adipose tissue from obese individuals with insulin resistance may help improve the metabolic perturbations observed in these patients.

Subjects and Methods

Study selection and sample processing

Subjects were recruited by the endocrinology and surgery departments at the University Hospital Joan XXIII (Tarragona, Spain) and University Hospital Virgen de la Victoria (Málaga, Spain) in accordance with the Helsinki Declaration. The corresponding hospital ethics committees responsible for research approved the study, and informed consent for biobanking surgically removed tissue was obtained from all participants. All patients had fasted overnight before collection of blood and adipose tissue samples. Visceral adipose tissue (VAT) and sc adipose tissue (SAT) samples from the same individual were obtained during the surgical procedure. Biobank samples from a nonmorbid (control) population were obtained after surgical procedures that included laparoscopic surgery for hiatus hernia repair or cholecystectomy. Samples from morbid obese subjects were obtained during bariatric surgery. Samples were collected from subjects according to stratification by age, gender, and BMI. Subjects were classified by BMI according to the World Health Organization criteria. The first cohort was selected at the Joan XXIII University Hospital. A second cohort was recruited at the Virgen de la Victoria University Hospital, Málaga. In this cohort, obese subjects were stratified according to their HOMA-IR as high insulin resistance (HOMA-IR >3.2) or low insulin resistance (HOMA-IR <3.2) as previously described (19). Using these criteria, 16 low-IR and 17 high-IR patients participated. For protein expression analysis, a third cohort from the Joan XXIII University Hospital was selected (18 women and 21 men, aged 59.9 ± 2.3 years and BMI range 19.6–56.8 kg/m^2). All subjects included in the 3 cohorts were of Caucasian origin and reported that their body weight had been stable for at least 3 months before the study. They had no systemic disease other than obesity, and all had been free of infection in the previous month before the study. Primary liver disease, cardiovascular disease, arthritis, acute inflammatory disease, infectious disease, and neoplastic and renal diseases were specifically excluded by biochemical evaluation. Patients on lipid-lowering drugs were excluded from this study.

In vitro cell culture

The SGBS preadipocyte cell line, provided by Dr Wabitsch (University of Ulm, Germany), was used as a cellular model of human sc adipocytes and was differentiated as described (20, 21). The hADSCs were isolated from the adipose tissue of 8 patients (3 lean, BMI 22.5 ± 0.3; 5 obese, BMI 30.8 ± 0.4) following published protocols (22). Fully differentiated cells were subjected to hypoxia for 24 hours in a modular incubator, which was flushed with 2% O_2, 93% N_2, and 5% CO_2. Control cells were cultured in a standard incubator (21% O_2 and 5% CO_2). Cells were lysed in RIPA buffer containing a protease inhibitor cocktail (Sigma-Aldrich), and protein concentration was determined with the bicinchoninic acid protein assay kit (Pierce).

Gene expression analysis

Total RNA was extracted from adipose tissue/cells using the RNeasy lipid tissue midi kit (QIAGEN Science). One microgram of RNA was reverse transcribed with random primers using the reverse transcription system (Applied Biosystems). Quantitative gene expression was evaluated in the first cohort with TaqMan

low-density arrays (Applied Biosystems; microfluidic cards). Samples from the second cohort were analyzed by real-time PCR on a 7900HT fast real-time PCR system using TaqMan gene expression assays (Applied Biosystems). Results were calculated using the comparative cycle threshold (Ct) method ($2^{-\Delta\Delta Ct}$) and expressed relative to the expression of the housekeeping genes cyclophilin 1A (*PPIA*) and *18S*.

Glucose transport

After treatments, cells were stimulated for 30 minutes with insulin, and glucose uptake was measured during the last 10 minutes of culture by incorporation of 2-deoxy-D[1-^{3}H]glucose as described (23). Glucose uptake rates were calculated as picomoles glucose taken up per 10 minutes per milligram protein, and results are expressed as the percentage of stimulation over basal (control = 100).

Statistical analyses

Statistical analysis was performed with SPSS version 15. For in vitro data, statistical significance was tested with the unpaired Student's *t* test or with a one-way ANOVA followed by the protected least-significant difference test. Differences in clinical variables, laboratory parameters, or expression variables between groups were compared by using nonparametric tests. Interactions between factors as well as the effects of covariates and covariate interactions with factors were assessed by Pearson's correlation analysis and general linear model univariate analysis. Correction for confounding and interacting variables was performed using stepwise multiple linear regression analysis.

Other methods were performed as specified in the Supplemental Appendix (published on The Endocrine Society's Journals Online website at http://jcem.endojournals.org).

Results

Expression of the GIPR is reduced in obese adipose tissue

We analyzed GIPR expression in adipose tissue from 3 independent cohorts. Clinical and laboratory data for the participants in cohorts 1 and 2 are summarized in Table 1. A significant reduction in GIPR mRNA expression was detected in the SAT depot (Figure 1A, $P < .01$) but not the VAT depot (Figure 1B) from obese subjects in cohort 1. Analysis of GIPR expression in a second cohort (cohort 2) confirmed this result and demonstrated significant reduction of GIPR expression in the SAT depot from obese subjects (Figure 1C; $P = .0015$) but not in the VAT depot (Figure 1D). Obese subjects from cohort 2 were then classified according to their insulin resistance, assessed by HOMA-IR. Interestingly, obese patients with high HOMA-IR values (>3.2) showed a significant decrease in GIPR gene expression in SAT depots compared with patients with low values (Figure 1E; $P = .0003$). Conversely, GIPR expression in the SAT or VAT depots of lean subjects was not dependent on the HOMA-IR value (data not shown). We also measured GIPR mRNA levels in stromal-vascular fraction (SVF) and adipocyte fraction obtained from visceral and sc fat depots. Although expression levels were tissue-dependent, GIPR mRNA could be detected in both SVF and adipocytes from sc and visceral regions (Figure 1F).

To further confirm these results, we performed protein expression studies in a third independent cohort of subjects. In good agreement with mRNA expression data, morbid obese subjects exhibited a significant reduction in GIPR protein expression in the SAT depot, as compared with overweight and obese subjects (Figure 2A; $P = .02$). In contrast, no differences in protein expression were found in the VAT depot from this group of patients (Figure 2B). Furthermore, morbid obese patients with a high IR value had decreased levels of GIPR protein (Figure 2C), which again was consistent with the transcription data from a separate cohort (Figure 1E). Immunofluorescence

Table 1. Anthropometric and Biochemical Variables in Cohorts 1 and 2 Used for mRNA Expression Levels[a]

	First Cohort		Second Cohort	
	Lean	Obese	Lean	Obese
n	19	37	10	38
Sex (male/female)	13/6	22/15	5/5	16/22
Age, y	51.68 ± 15.96	58.58 ± 13.21	42.90 ± 10.70	41.97 ± 11.91
BMI, kg/m^2	23.11 ± 1.59	29.07 ± 3.02[b]	23.29 ± 1.33	42.39 ± 14.57[b]
Waist, cm	83.7 ± 7.9	99.29 ± 14.33[b]	83.15 ± 6.78	116.22 ± 25.5[b]
DBP, mm Hg	68.47 ± 9.90	76.4 ± 11.77[c]	78.80 ± 19.62	79.14 ± 12.98
SBP, mm Hg	121.47 ± 11.77	135.84 ± 16.90[c]	110.10 ± 21.94	131.11 ± 18.26[b]
Glucose, mmol/L	4.33 ± 0.68	5.56 ± 0.49[b]	4.77 ± 0.63	5.19 ± 0.63
Cholesterol, mmol/L	5.19 ± 1.18	5.10 ± 0.95	5.32 ± 1.06	5.12 ± 1.01
HDLc, mmol/L	1.46 ± 0.21	1.35 ± 0.29	1.52 ± 0.39	1.26 ± 0.36
Triglycerides, mmol/L	1.15 ± 0.53	1.17 ± 0.66	1.11 ± 0.58	1.45 ± 0.69
Insulin, μIU/mL	4.52 ± 3.44	6.71 ± 4.94	6.75 ± 2.67	15.87 ± 11.56[b]
HOMA-IR	1.11 ± 0.84	1.74 ± 1.35	1.40 ± 0.48	3.81 ± 2.83[c]

Abbreviation: HDLc, high-density lipoprotein cholesterol.

[a] Results are given as mean ± SD.

[b,c] Significant differences between the means of the different groups: [b] $P < .001$; [c] $P < .01$ (Student's *t* test).

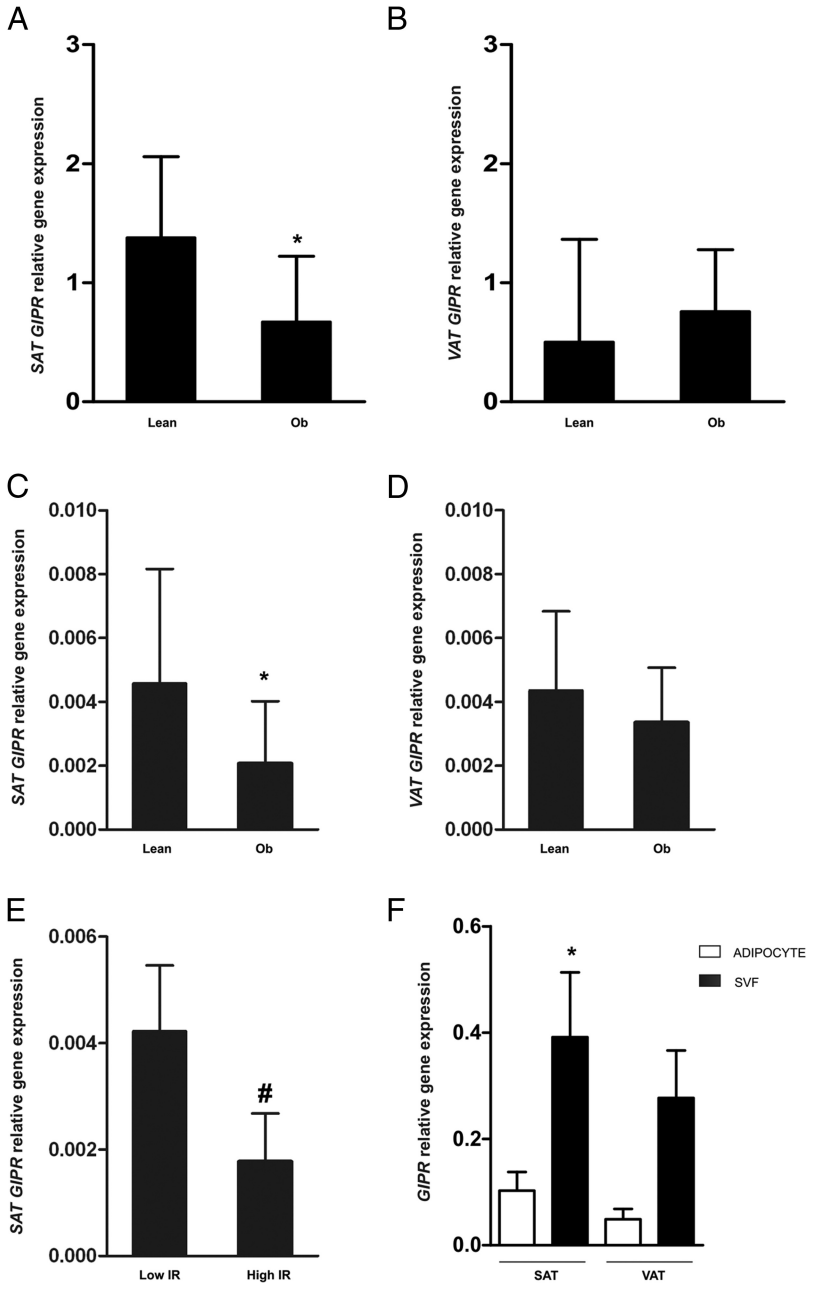

Figure 1. GIPR mRNA expression in human adipose tissue from lean and obese (Ob) subjects. A and B, Subjects from the first cohort (n = 48) were divided into 2 groups according to BMI (lean, BMI <25 kg/m^2; obese, BMI >25 kg/m^2). C and D, Subjects from the second cohort were divided as in A and B. E, Obese subjects from the second cohort were classified according to insulin resistance assessed by HOMA-IR (low IR, HOMA-IR <3.2; high IR, HOMA-IR >3.2). F, SVF and isolated adipocytes (n = 6; paired adipose biopsies) from SAT and VAT. GIPR mRNA expression was determined by quantitative PCR. Data comparisons were made by Kruskal-Wallis test for multiple groups and Mann-Whitney *U* test for 2 groups. Data are presented as median with interquartile range. Significant differences: *, $P < .01$ vs lean; #, $P < .001$ vs obese with low IR.

detection of GIPR in SAT depots confirmed the difference in GIPR protein expression associated with obesity (Figure 2D). Thus, although the SVF could be considered as the main contributor of GIPR expression in adipose tissue (Figure 1F), GIPR is clearly expressed on the surface of human mature adipocytes (Figure 2D). Finally, Western blot analysis revealed a decrease in GIPR protein levels in both SVF and mature adipocytes in obese compared with lean subjects (Figure 2E). Collectively, these results suggest that a decrease in GIPR expression in SAT is associated with increased insulin resistance in the context of obesity.

Insulin resistance influences GIPR gene expression in the SAT depot

In bivariate analysis, GIPR gene expression levels in the SAT of cohort 1 participants correlated negatively with BMI, waist circumference, systolic blood pressure (SBP), and blood glucose and triglyceride levels (Table 2). No clinical or anthropometric associations were observed regarding VAT gene expression. To strengthen the independence of these associations as predictors of GIPR gene expression, a multiple regression analysis model was constructed for each depot including the above-mentioned bivariate correlations, adjusting for age and gender. BMI, waist circumference, SBP, glucose, triglyceride and HOMA-IR index were selected as independent variables in both SAT and VAT depot models. SAT GIPR expression was predicted by SBP (B [unstandardized regression coefficient] = −0.045, $P = .01$). Interestingly, HOMA-IR (B = −0.365, $P = .003$) and blood glucose (B = −0.616, $P = .049$) emerged as major determinants of GIPR expression in SAT from obese subjects (BMI >30 kg/m^2). In the VAT depot, GIPR was predicted only by SBP (B = −0.041, $P = .027$). A similar analysis was performed on the second cohort. In this study, GIPR gene expression in SAT showed a strong negative correlation with BMI, waist circumference, insulin, and HOMA-IR and a positive correlation with adiponectin (Table 2). Similar correlations were found for insulin, HOMA-IR, and adiponectin in VAT (Table 2). Consistent with the results obtained in cohort 1, multiple regression analysis of cohort 2 adjusting for age and gender revealed HOMA-IR as a variable strongly associated with GIPR expression in SAT (B = −0.535, $P = .007$) (Table 2). Additionally, VAT GIPR ex-

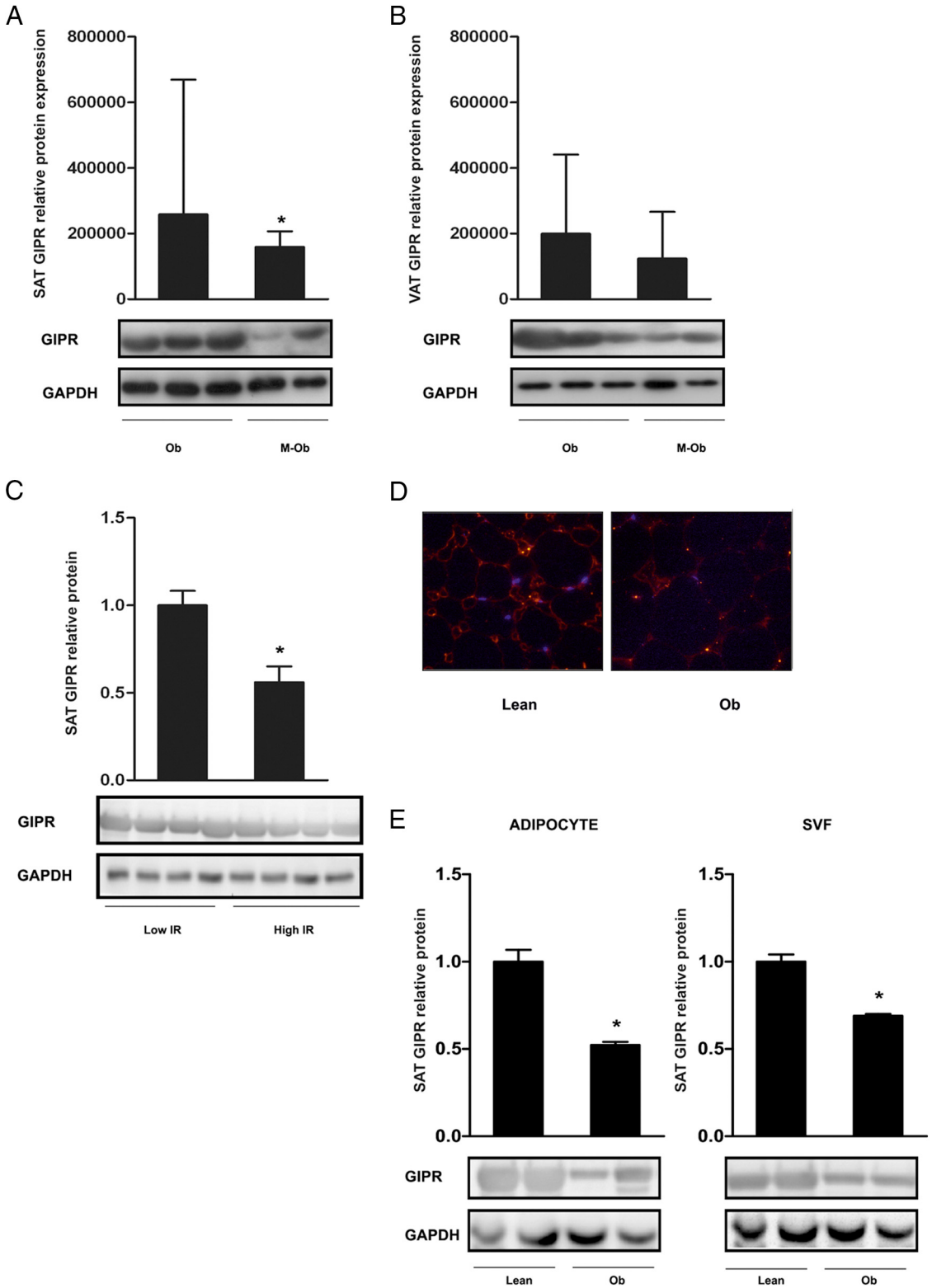

Figure 2. GIPR protein expression in human adipose tissue of obese individuals. A and B, GIPR protein expression was analyzed by Western blot in SAT (A) and VAT (B) from 39 subjects with a BMI range of 19.6 to 56.8 kg/m^2. Subjects were divided into 2 groups (obese [Ob], BMI between 25 and 40 kg/m^2 and morbidly obese [M-Ob], BMI >40 kg/m^2). GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as a loading control. Representative Western blot and densitometric analysis is shown. Data comparisons were made by Mann-Whitney *U* test, and data are presented as median with interquartile range. Significant differences: *, $P < .05$ vs obese with low IR. C, GIPR protein expression was determined in adipose tissue of morbidly obese patients with low vs high IR classified as in Figure 1E. Representative Western blot and densitometric analysis is shown. D, Immunofluorescence of GIPR (red) in SAT from lean (BMI <25 kg/m^2) and obese (BMI >30 kg/m^2) subjects. Nuclei were stained with 4′,6-diamidino-2-phenylindole (blue). E, GIPR protein expression was analyzed by Western blot in mature adipocytes and SVF isolated from adipose tissue of lean (n = 3–5) and obese (n = 4–5) subjects. Significant differences: *, $P < .05$ vs control

Table 2. Bivariate Correlations Between *GIPR* and Anthropometric, Metabolic, and Gene Expression Analysis in the Whole Population (Cohorts 1 and 2)

	First Cohort				Second Cohort			
	SAT GIPR		VAT GIPR		SAT GIPR		VAT GIPR	
	R	*P*	*R*	*P*	*R*	*P*	*R*	*P*
BMI	−0.390	.003	NS	NS	−0.490	.001	NS	NS
SBP	−0.366	.02	NS	NS	NS	NS	NS	NS
Waist	−0.352	.01	NS	NS	−0.421	.006	NS	NS
Glucose	−0.273	.04	NS	NS	NS	NS	NS	NS
Triglyceride	−0.338	.013	NS	NS	NS	NS	NS	NS
Insulin	NS	NS	NS	NS	−0.467	.001	−0.296	.041
HOMA-IR	NS	NS	NS	NS	−0.526	.0001	−0.294	.042
Adiponectin	NS	NS	NS	NS	0.598	.002	0.572	.003

Abbreviation: NS, not significant.

pression was positively determined by adiponectin (B = 0.610, $P = .001$). Taken together, our in vivo observations suggest that insulin sensitivity might predict GIPR expression in SAT.

GIP improves insulin sensitivity in human adipocytes

Given the results above, we analyzed the potential modulation of insulin-sensitive glucose uptake by GIP in human adipocytes. We found that chronic (24 hours) but not short-term (6 hours) exposure to GIP resulted in a significant increase in insulin-induced glucose uptake in mature sc adipocytes (Figure 3A). A similar effect was found for human visceral adipocytes (Supplemental Figure 1A) and myocytes (Supplemental Figure 1B), suggesting that, with respect to glucose uptake, GIP could be considered an insulin-sensitizer incretin acting independently of tissue type. Additionally, insulin stimulation of insulin receptor substrate 1 (IRS1), AKT, and ERK1/2 phosphorylation were all significantly increased in human sc adipocytes treated with GIP (Figure 3B).

Analogous to other G protein-coupled receptors (GPCRs), studies in pancreatic cells have shown that the activity of GIPR is regulated by GPCR kinase 2 (GRK2), which promotes its internalization (24). When GRK2 mRNA levels were assessed in cohort 1, we found a direct relationship with GIPR expression in SAT; indeed, it was one of the strongest determinants of GIPR expression in this depot (B = 2.744; $P < .0001$). In addition to its regulation of GPCR activity, GRK2 also participates in the control of several members of the receptor-tyrosine kinase family, including the insulin receptor (25). Specifically, GRK2 may act as an inhibitor of insulin signaling through a mechanism based on the formation of dynamic GRK2/IRS1 complexes (26). In an attempt to determine a molecular contribution to the insulin-sensitizing activity of GIP, coimmunoprecipitation experiments with GRK2 were performed in human sc adipocytes responding to GIP. As anticipated, GIP stimulation induced an association of GRK2 with GIPR in adipocytes (Figure 3C). Concurrently, GIP treatment also resulted in a partial, but significant, decrease in the interaction between GRK2 and IRS1 (Figure 3C). This dynamism between GRK2 and IRS1 upon GIP stimulation may thus prompt an insulin-sensitizing effect.

Hypoxia decreases GIP sensitivity of human sc adipocytes

During the progression to obesity, adipose tissue is one of the first organs affected by insulin resistance, which is closely associated with local hypoxia (27). To determine whether the response to GIP in human adipocytes could be compromised by poor oxygenation, insulin-induced glucose uptake and signaling in the presence of GIP was measured in sc adipocytes cultured in normoxia (21% O_2) or mild hypoxia (2% O_2). Compared with control conditions, the insulin-sensitizing effect of GIP on glucose uptake was lost in sc adipocytes under hypoxia (Figure 3D). Moreover, compared with normoxic conditions, insulin-induced phosphorylation of IRS1 and AKT was not enhanced by GIP treatment under hypoxia (Figure 3E). Cell viability was not significantly affected by culture in low O_2 (data not shown), suggesting that the loss of GIP sensitivity is not a consequence of cell death. Overall, these results point to a GIP-resistant phenotype occurring in human adipocytes, in which hypoxia mimics an obese environment.

The response to GIP is blunted in human adipocytes from obese patients

Our results thus far suggested that the response to GIP may be blunted under pathological conditions. Thus, we next evaluated glucose uptake in adipocytes differentiated from hASDCs obtained from lean and obese patients.

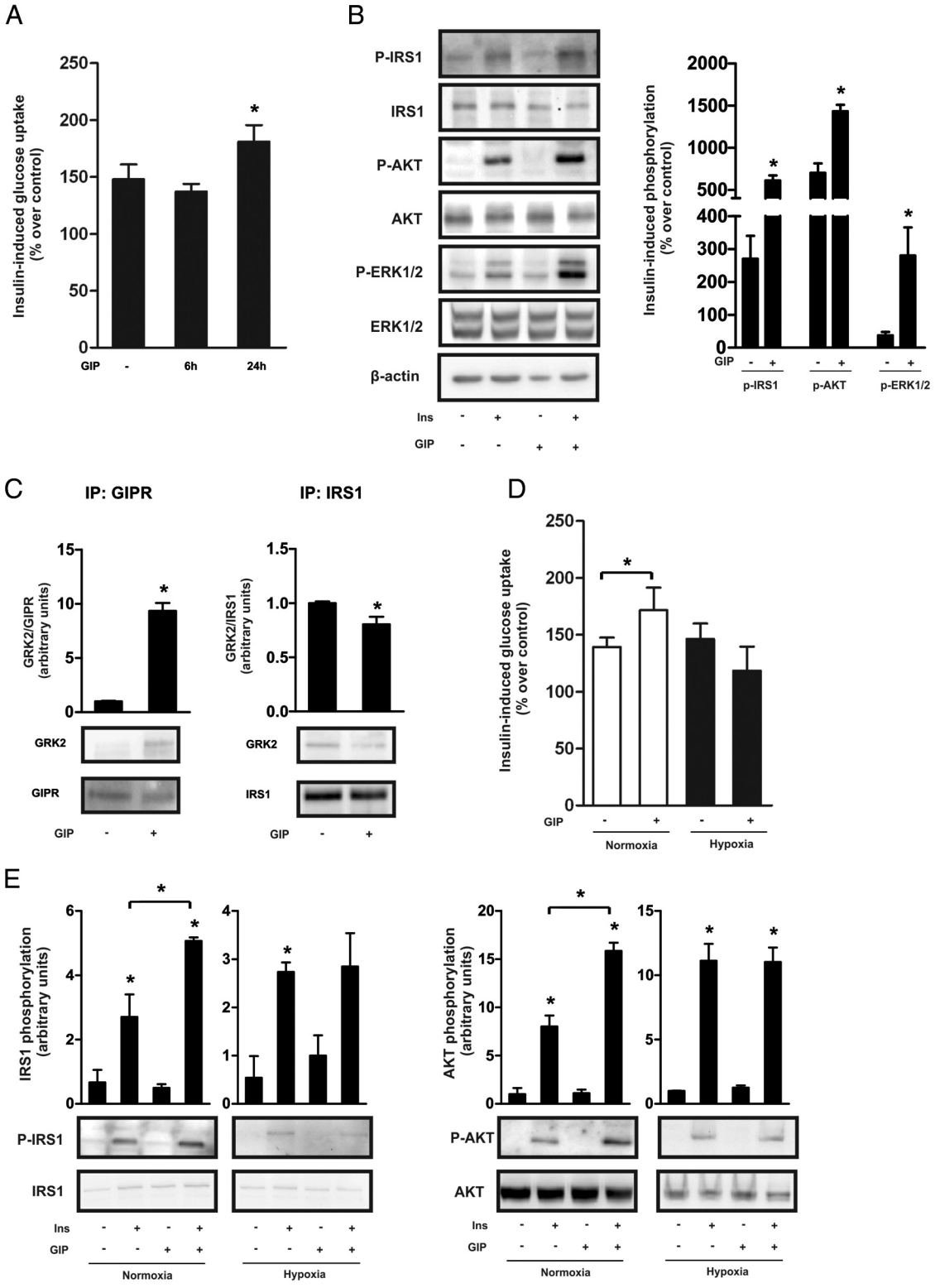

Figure 3. GIP modulates insulin sensitivity, but hypoxia reduces the beneficial effects of GIP in human sc adipocytes. A, Differentiated SGBS cells were incubated with GIP (100nM) for 6 and 24 hours. After treatment, cells were incubated with 100nM insulin for 30 minutes, and glucose uptake was measured during the last 10 minutes by quantification of 2-deoxyglucose incorporation. Results are mean and SEM from 3 independent experiments performed in triplicate. Data are expressed as percentage of stimulation over basal (control = 100). Significant differences: *, $P < .01$ vs control (no GIP). B, Lysates from differentiated SGBS cells cultured in the absence or presence of 100nM GIP for 24 hours before stimulation with 100nM insulin (Ins) for 15 minutes were analyzed by Western blot using antibodies against phosphorylated and total IRS1 (Tyr612), Akt (Ser473), ERK1/2 (Thr202/Tyr204), and p70S6K1 (Thr389). A representative experiment is shown together with densitometric analysis of phosphorylated vs total proteins (3 independent experiments). C, Total cell lysates from differentiated SGBS cells cultured in the absence or presence of 100nM GIP for 24 hours were immunoprecipitated with anti-GIPR or anti-IRS1 antibodies, followed by immunoblotting with the (*Continued*)

Consistent with the insulin-sensitizing effect found in the adipose tissue cell line (Figure 3, A and B), GIP treatment for 24 hours resulted in a significant increase in insulin-induced glucose uptake in adipocytes derived from lean patients (Figure 4A). Notably, this response was lost in obese-derived adipocytes (Figure 4A). We observed a similar GIP-stimulated increase in insulin-induced LPL activity in adipocytes obtained from lean, but not obese, subjects (Figure 4B). Furthermore, the sensitizing effect of GIP on AKT phosphorylation induced by insulin in lean adipocytes was blunted in obese-derived adipocytes (Figure 4C). Given the known participation of the incretin-degrading enzyme DPP4 in glucose metabolism, together with its known expression in adipocytes (28), potential differences in DPP4 expression were considered. In mature adipocytes from lean patients, GIP treatment led to a reduction in the level of DPP4 mRNA (Figure 4D), which was accompanied by an increase in GIPR expression (Figure 4E). Although expression of both DPP4 and GIPR was also detected in adipocytes from obese patients, GIP treatment failed to induce their differential expression (Figure 4, D and E). Importantly, no significant differences were found in the differentiation capacity of lean- and obese-derived adipocytes, as measured by expression of the mature markers adiponectin and peroxisome proliferator-activated receptor-γ (Supplemental Figure 2). However, analysis of GIPR mRNA (Figure 4F) and protein (Figure 4G) expression revealed that the GIP-resistant phenotype observed in obese-derived adipocytes might result from a decrease in GIPR expression. Collectively, these results demonstrate that the ability of adipocytes to respond to GIP depends upon the environment from where they are isolated.

Discussion

Incretin hormones such as GIP act to promote efficient uptake and storage of energy after food ingestion and have become important players for glucose homeostasis in pancreatic and extrapancreatic tissue. Indeed, GIPRs can be found in peripheral organs such as adipose tissue (18) and could function to regulate adipocyte metabolism. Here we demonstrate a defective GIP/GIPR signaling axis in obese-derived adipose tissue, which is characterized by decreased GIPR expression in the sc adipose depot, an inverse relationship to insulin resistance, and a GIP-resistant phenotype in obese-derived adipocytes. Interestingly, although there is a clear link established between VAT and metabolic complications such as T2D, dyslipidemia, and hypertension, the fact that our major findings were detected in the SAT depot would be in agreement with the hypothesis that a primary defect in sc fat might be considered as a causal link between obesity and insulin resistance (29).

Although circulating levels of GIP are usually increased in patients with T2D, the insulinotropic activity of GIP is blunted under these conditions (3). Other findings suggest that alterations in GIPR expression in SAT might be associated with signs of insulin resistance in nondiabetic women with central obesity (18). Also, a recent report indicates that GIPR expression in SAT is inversely associated with BMI (30). Our findings, in 3 independent cohorts, confirm and extend this recent analysis by providing a detailed analysis of clinical variables associated with an insulin-resistant metabolic profile including BMI, SBP, waist circumference, and glucose levels. It is worth noting that although pooling results from all cohorts might increase the power and sensitivity of our study, the inclusion of cohorts phenotypically different might be more reflective of real populations. In this regard, cohort 1 included a high percentage of overweight subjects (44%), whereas the cohort 2 comprised morbidly obese patients (40%). Furthermore, our results complement the observation that hyperglycemia leads to a downregulation of GIPR expression in pancreatic β-cells, and normalization of glucose levels in patients with poorly controlled T2D enhances the incretin response of GIP (31). Interestingly, we found that with obese subjects, insulin resistance persists as an important determinant of GIPR expression in sc fat, independent of BMI. Thus, obese patients with the highest degree of insulin resistance exhibit the lowest levels of GIPR. Taken together, these observations support a link between GIP and insulin sensitivity in adipose tissue. Indeed, our results indicate that the relationship between obesity and GIPR expression might be mediated through the degree of insulin resistance. Accordingly, there are no significant differences in the expression of GIPR mRNA between lean and obese patients with low insulin resistance (data not shown), suggesting that low levels of GIPR

Figure 3 (Continued). corresponding antibodies against GRK2, IRS1, or GIPR. A representative immunoblot of 2 independent experiments and the corresponding densitometric analysis is shown. D, Fully differentiated SGBS cells were placed in a hypoxic environment for 24 hours or cultured in a standard incubator as described. Cells were then cultured for 24 hours in the absence or presence of 100nM GIP. Insulin-induced glucose uptake was determined, and results from 3 independent experiments performed in triplicate are expressed as the percentage of stimulation over basal (control = 100). E, Lysates from differentiated adipocytes cultured as in D before stimulation with 100nM insulin for 15 minutes were analyzed by Western blot using antibodies against phosphorylated and total IRS1 (Tyr612) and AKT (Ser473). Representative immunoblots of 2 to 3 independent experiments and densitometric analysis are shown. Significant differences: *, $P < .05$ vs control.

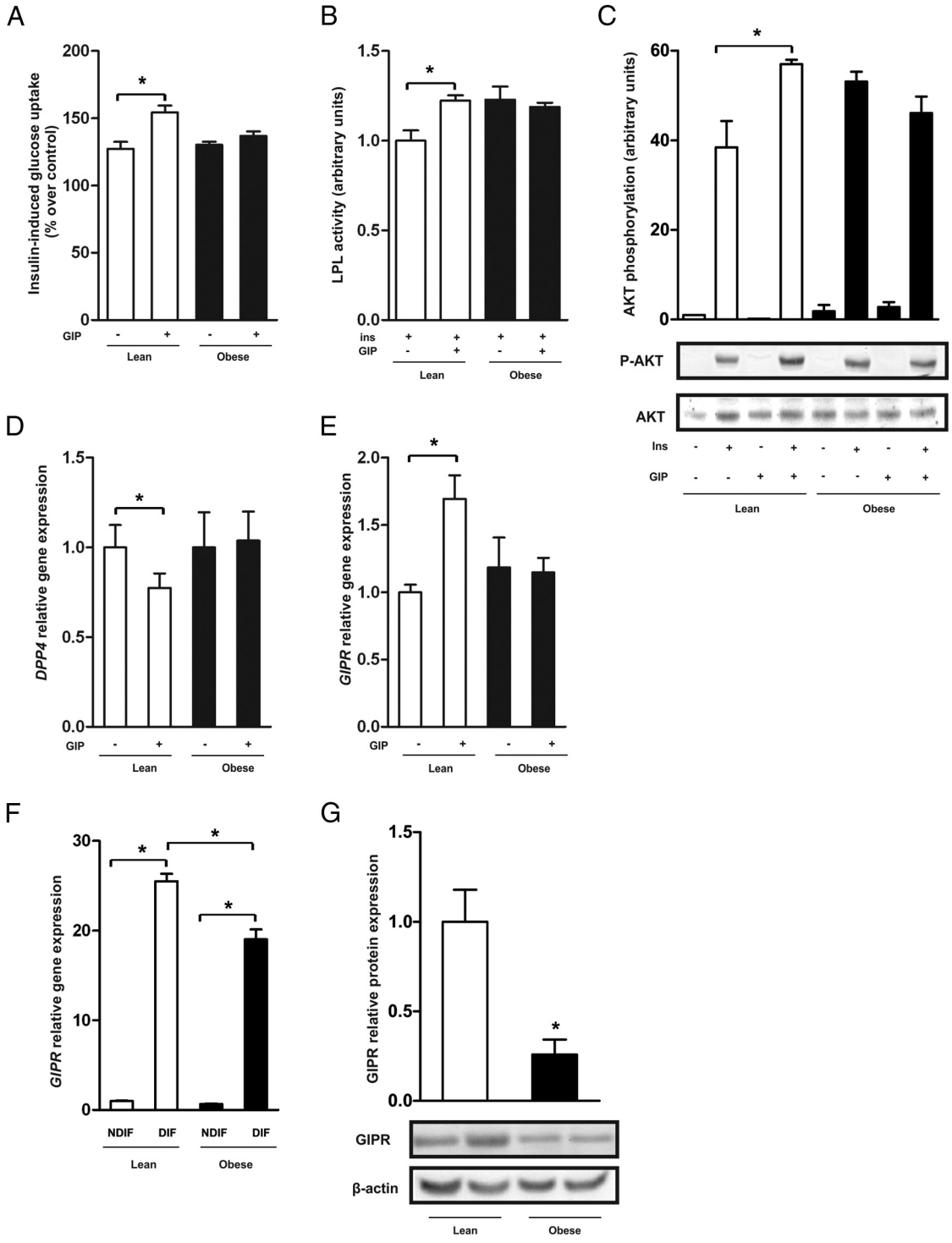

Figure 4. Adipocytes differentiated from adipose stem cells of obese subjects have a GIP-resistant phenotype. hADSCs were obtained from lean and obese subjects as described. After differentiation, cells were incubated in the absence or presence of 100nM GIP for 24 hours. A, Glucose uptake was measured after addition of 100nM insulin for 30 minutes by incorporation of 2-deoxyglucose into the cells during the last 10 minutes of culture. Results from 3 independent experiments performed in triplicate are expressed as the percentage of stimulation over basal (control = 100). Significant differences: *, $P < .05$ vs control. B, LPL activity in human adipocytes obtained from lean and obese subjects was analyzed after GIP treatment followed by incubation with 100nM insulin (Ins) for 24 hours. Data are presented as mean ± SEM. Significant differences: *, $P < .05$ vs control. C, Lysates from differentiated adipocytes cultured for 24 hours in the absence or presence of 100nM GIP before stimulation with 100nM insulin for 15 minutes were analyzed by Western blot using antibodies against phosphorylated and total AKT (Ser473). A representative experiment is shown together with densitometric analysis of phosphorylated vs total proteins (2 independent experiments). D and E, DPP4 (D) and GIPR (E) mRNA expression was analyzed by quantitative PCR in lean and obese mature adipocytes after treatment with 100nM GIP for 24 hours. F and G, GIPR mRNA (F) and protein (G) expression in hADSCs obtained from lean (n = 3–5) and obese (n = 3–5) patients. Data are presented as mean ± SEM. Significant differences: *, $P < .01$ vs lean control.

detected in obese patients might be directly associated with the loss of insulin sensitivity clearly associated with weight gain.

Adipocytes are key regulators of systemic carbohydrate and lipid homeostasis and are essential for the control of energy balance. Adipose tissue is also a central player in the mild inflammatory state characteristic of obesity and is one of the first tissues affected by insulin resistance. Our study reveals GIP as an insulin-sensitizer incretin during glucose uptake, working in a manner similar to that described for lipid metabolism (10, 11). Analogous to other incretin receptors, such as GLP-1R (32), GIPR is largely expressed in cells from the SVF. However, the reduced expression of GIPR in adipocytes does not necessarily indicate the lack of a significant role for this receptor in adipose tissue biology. Our study reveals GIP as an insulin-sensitizer incretin in mature adipocytes but does not exclude additional functions for this incretin in other components of adipose tissue. As stated previously, the effects of GIP on insulin sensitivity is a controversial subject in the literature (12, 13, 15). Indeed, our findings in human adipocytes are at odds with those of Timper and colleagues (17) who detected no difference in the response to GIP or insulin from adipocytes obtained from donors differing in their obese and diabetic phenotype. Recent data, however, demonstrate that an altered metabolism is a characteristic feature of hADSCs isolated from obese tissue (33). In line with these findings, we observed an insulin-sensitive phenotype in response to long-term treatment with GIP in adipocytes obtained from lean donors but, conversely, a GIP-resistant phenotype in adipocytes obtained from hADSCs of obese subjects. Differences in the nature of the progenitor cell (preadipocyte vs hADSC), or in the differentiation medium used, could explain the discrepancies observed between our study and that of Timper et al (17). Our findings using lean and obese adipocytes are strengthened by the observation that decreased GIP responsiveness occurred in an adipocyte cell line cultured in hypoxia to mimic the obese milieu. Moreover, the GIP-resistant phenotype we detected on insulin sensitivity was observed also when we analyzed other GIP properties at the transcriptional level, including inhibition of IL-6 expression (data not shown). Furthermore, we demonstrate for the first time that GIP, which is a known endogenous substrate for DPP4, decreases mRNA expression of this dipeptidase. We therefore hypothesize that GIP might potentiate its effects by increasing GIPR expression, and also decreasing DPP-4 expression, thus preventing its own deactivation. Interestingly, DPP4 has been recently uncovered as a novel biomarker in adipose tissue and may provide a link between obesity and the metabolic syndrome (28). Our data describing the beneficial effects of GIP treatment on insulin sensitivity are similar to the effects described for GLP-1, which also appears to improve insulin sensitivity and glucose uptake (34, 35). It should be stated, however, that the effect of GIP on insulin sensitivity in human adipocytes in vitro was observed after relatively long-term treatment (24 hours), whereas physiological effects of GIP in vivo occur in the postprandial period. Reconciling these differences is challenging, although most studies that examine GIP use comparable in vitro conditions (17, 36, 37), and little is known regarding the in vivo actions of GIP in human adipose tissue.

GPCR-mediated modulation of the insulin signaling pathway has been demonstrated in several cellular models, including adipose tissue (25). Specifically, GRK2, which promotes GPCR desensitization, might also reduce the ability of IRS1 to associate with the insulin receptor, leading to an inhibition of insulin action (26). Our results provide the first demonstration of an interaction between GRK2 and GIPR in human adipocytes, in a manner similar to that described previously in pancreatic islet cells (24). Excitingly, multiple regression analysis has uncovered GRK2 as one of the main determinants of GIPR mRNA expression in SAT depots in both lean and obese subjects. Accordingly, our findings support a model whereby GIP interaction with GIPR modulates the shuttling of GRK2 to IRS1, which leads to an increase in insulin action in adipocytes.

In conclusion, our study defines a key role for the GIPR/GIP signaling axis in the pathogenesis of obesity and associated insulin resistance. GIP has important metabolic actions other than its physiological glucose-dependent incretin effect. Particularly in human adipose tissue, GIP might be acting as an insulin sensitizer and an anti-inflammatory factor. Despite the fact that some animal data point to GIPR inhibition as a potential treatment for obesity (5–8), this work is somewhat controversial because beneficial effects for GIP have also been described in mice (9). It is becoming clear that important differences in metabolic and signal transduction exist between rodent and human adipose tissues. Thus, caution should be exercised when extrapolating information from one species to another. Our data from clinical studies, together with results from in vitro experiments, propose that insulin-resistant states related to obesity might be associated with diminished GIP sensitivity. Hence, GIPR expression in SAT might determine systemic insulin sensitivity. Indeed, our studies with hADSCs obtained from obese donors point to a GIP-resistant phenotype as a possible predisposing genetic factor contributing to the development of clinical glucose intolerance and insulin resistance. Consistent with this hypothesis, regulation of GIPR expression by epigenetic mechanisms such as histone acetylation has been re-

cently reported (38), and the plasticity of the DNA methylation pattern has been revealed as an important player in lineage commitment of hADSCs (39). Further investigations will be required to determine whether the failure of obese adipose tissue to properly respond to GIP is consequence of an altered epigenetic regulation of GIPR, originating from the obese environment.

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