Hepatocyte Growth Factor Induces Glucose Uptake in 3T3-L1 Adipocytes through A Gab1/Phosphatidylinositol 3-Kinase/Glut4 Pathway*

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Adipose tissue is a source of hepatocyte growth factor (HGF), and circulating HGF levels have been associated with elevated body mass index in human. However, the effects of HGF on adipocyte functions have not yet been investigated. We show here that in 3T3-L1 adipocytes HGF stimulates the phosphatidylinositol (PI) 3-kinase-dependent protein kinase B (PKB) activity, AS160 phosphorylation, Glut4 translocation, and consequently, glucose uptake. The initial steps involved in HGF- and insulin-induced glucose uptake are different. HGF enhanced the tyrosine phosphorylation of Gab1, leading to the recruitment of the p85-regulated subunit of PI 3-kinase, whereas p85 was exclusively recruited by IRS1 in response to insulin. In adipocytes rendered insulin-resistant by a long-lasting tumor necrosis factor α treatment, the protein level of Gab1 was strongly decreased, and HGF-stimulated PKB activation and glucose uptake were also altered. Moreover, treatment of 3T3-L1 adipocytes with thiazolidinedione, an anti-diabetic drug, enhanced the expression of both HGF and its receptor. These data provide the first evidence that in vitro HGF promotes glucose uptake through a Gab1/PI 3-kinase/PKB/AS160 pathway which was altered in tumor necrosis factor α-treated adipocytes.

White adipose tissue is the main site of energy storage; glucose and free fatty acids are transported into the adipocytes and stored as triacylglycerols. Glucose transport is largely induced by insulin, which stimulates the translocation of the glucose transporters Glut 4 and to a lesser extent Glut 1 from an intracellular pool to the plasma membrane (1). This biological response requires tyrosine phosphorylation of IRS1, which in turn leads to binding and activation of PI 3-kinase. Downstream effectors of PI 3-kinase such as protein kinase B (PKB)2 and its substrate AS160 are involved in Glut 4 translocation. Furthermore, insulin-induced Glut 4 translocation could also involve the activation of a second pathway, which is completely independent of PI 3-kinase activity; that is, the Cbl/Crk-II/C3G/TC10 pathway (2–5).

Obesity is highly associated with metabolic disorders including insulin resistance. The insulin resistance of the adipose tissue could be involved in general insulin resistance and liver complications of obesity (6–9). White adipose tissue might be implicated in these complications through the secretion of a number of bioactive peptides and proteins, generally known as adipokines. Indeed, white adipose tissue is a source of hormones, cytokines, acute phase proteins, and growth factors including epidermal growth factor, vascular endothelial growth factor, transforming growth factor, nerve growth factor, and also hepatocyte growth factor (HGF). In fact, HGF is expressed and secreted by mouse 3T3-L1 adipocytes (10) and human adipose tissue (11, 12). Furthermore, HGF levels have been reported to be elevated in obese patients and raised with body mass index (13). On the other hand weight loss after gastroplasty has been shown to be associated with a reduction of HGF plasma levels in obese patients (12, 14). A strong association between elevated serum HGF and metabolic syndrome has also been reported (15).

The role of the local production of HGF in adipocyte functions has not yet been investigated. The HGF/HGF receptor pathway is indirectly involved in glucose transport. HGF could regulate the expression of glucose transporters in β-cells (16) and after massive small bowel resection (17). HGF also enhances the glycolytic and oxidative phosphorylation pathways of energy production in murine mammary cancer cells (18). However, at present nothing is known about the metabolic role of HGF in adipocytes.

HGF mediates its effects via a specific receptor. The HGF receptor is a cell surface glycoprotein composed of one extracellular α-subunit and one transmembrane β-subunit. Binding of HGF to its receptor stimulates the tyrosine kinase activity of

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the β-subunit that leads to receptor autophosphorylation and to the membrane recruitment and tyrosine phosphorylation of intracellular signal transducers. Two-phosphorylated tyrosines (Tyr-1349 and Tyr-1356) in the C-terminal tail of the HGF receptor β-subunit bind multiple SH2-containing transducers such as PI 3-kinase, the adaptor protein Grb2, and the docking protein Grb2-associated binder 1 (Gab1) (19, 20). The latter provides an essential anchorage for phospholipase Cγ1, Crk-II, and PI 3-kinase (21, 22). The aim of the present study was to characterize the HGF effect on glucose transport in adipocytes, to compare this with the molecular mechanism of insulin-induced glucose uptake, and to look for modifications in insulin-resistant adipocytes.

EXPERIMENTAL PROCEDURES

Materials—Dulbecco’s Modified Eagle Medium (DMEM) was obtained from Cambrex Bio Sciences (Verviers, Belgium). Fetal calf serum and calf serum were from PAA Laboratories (Les Mureaux, France). Insulin was obtained from Lilly (Suresnes, France). HGF and TNFα were from PeproTech, Inc. (Rocky Hill, NJ). 2-[3H]Deoxyglucose was purchased from PerkinElmer Life Sciences. [γ-32P]ATP was purchased from GE Healthcare. Polyvinylidene difluoride membranes were purchased from Millipore (Bedford, MA). BCA reagent was obtained from Pierce. A protease inhibitors mixture was obtained from Roche Diagnostics. All other chemical reagents were purchased from Sigma. Antibodies against phosphoryrosine (clone 4G10), Gab1, and the p85 subunit of PI 3-kinase were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-conjugated and fluorescein isothiocyanate-conjugated secondary antibodies were obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). Enhanced chemiluminescence reagent was purchased from PerkinElmer Life Sciences.

Animals—Genetically obese diabetic db/db mice (n = 6) and their lean control db/+ littermates (n = 6) were purchased from Charles River Laboratories (St. Aubin les Elbeuf, France). Mice were housed at the animal facility of the Faculty of Medicine (Nice, France). Mice were maintained under a 12-h light: 12-h dark cycle and had free access to water and food. Mice were killed by cervical dislocation, and epididymal fat pads were removed and frozen-clamped in liquid nitrogen. Principles of laboratory animal care were followed, and the Ethical Committee of the Faculty of Medicine approved the animal experiments.

Cell Culture—3T3-L1 fibroblasts were grown in 35- or 100-mm dishes in DMEM, 25 mM glucose, and 10% calf serum and induced to differentiate into adipocytes as described previously (23). Briefly, 2 days after confluence, medium was changed for DMEM, 25 mM glucose, 10% fetal calf serum supplemented with isobutylmethylxanthine (0.5 mM), dexamethasone (0.25 μM), rosiglitazone (10 μM), and insulin (5 μg/ml). The medium was removed after 2 days and replaced with DMEM, 25 mM glucose, 10% fetal calf serum supplemented with rosiglitazone (10 μM), and insulin (5 μg/ml) for 2 other days. Then cells were fed every 2 days with DMEM, 25 mM glucose, 10% fetal calf serum. 3T3-L1 adipocytes were used 9–18 days after the beginning of the differentiation protocol. Before each experiment 3T3-L1 adipocytes were serum-starved overnight in DMEM supplemented with 0.5% bovine serum albumin.

2-Deoxyglucose Uptake—After serum starvation, 3T3-L1 adipocytes were incubated or not with wortmannin (100 nM) for 20 min or with TNFα (50 ng/ml) for 24 h. Cells were then washed with Krebs-Ringer phosphate buffer (10 mM phosphate buffer, pH 7.4, 136 mM NaCl, 4.7 mM KCl, 1.25 mM MgSO4) and incubated for 10 min in Krebs-Ringer phosphate buffer supplemented with 0.2% bovine serum albumin and 1.25 mM CaCl2. Then cells were either left untreated or stimulated with HGF or insulin (as indicated in figure legends) with or without wortmannin (100 nM) for 20 min. Glucose transport was determined by the addition of 2-[3H]deoxyglucose (0.1 mM, 0.5 μCi/ml) as described previously (23). The reaction was stopped after 3 min by aspiration, and cells were washed 3 times with ice-cold PBS. Cells were sonicated in PBS, and glucose uptake was assessed by scintillation counting. Results were normalized for protein content measured by BCA assay.

Western Blotting and Co-immunoprecipitation Assays—After serum starvation 3T3-L1 adipocytes were incubated or not with TNFα (50 ng/ml) for 24 h. 3T3-L1 adipocytes were then incubated with HGF (100 ng/ml) or insulin (0.5 nM) for different periods of time as indicated in the figure legends. Cells were subsequently washed twice with ice-cold buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 10 mM EDTA, 150 mM NaF, 2 mM sodium orthovanadate, and 10 mM pyrophosphate) before solubilization for 30 min at 4 °C in lysis buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 10 mM EDTA, 150 mM NaF, 2 mM sodium orthovanadate, 10 mM pyrophosphate, proteases inhibitors, and 1% Triton X-100). Lysates obtained after centrifugation (15 min at 15,000 × g at 4 °C) were incubated for 4 h at 4 °C with appropriate antibodies preadsorbed on protein-G-Sepharose. After washes with lysis buffer, immune pellets were resuspended in Laemmli buffer and boiled for 5 min. Proteins were separated by SDS-PAGE using a 7.5% resolving gel and transferred to a polyvinylidene difluoride membrane. The membrane was blocked with saline buffer (10 mM Tris, pH 7.4, 320 mM NaCl) containing 4% bovine serum albumin for 1 h at room temperature and blotted overnight at 4 °C with the indicated antibodies at the dilution indicated by the manufacturer’s instructions. After incubation with horseradish peroxidase-conjugated secondary antibodies, proteins were detected by enhanced chemiluminescence. In some cases the membrane was stripped for 30 min at 50 °C in 62 mM Tris, pH 6.7, 100 mM 2-mercaptoethanol, and 2% SDS and reprobed with the indicated antibodies.
Measurement of PI 3-Kinase Activity—After serum starvation 3T3-L1 adipocytes were either left untreated or incubated with HGF (100 ng/ml) for 10 min. Cell lysates were successively immunoprecipitated with anti-Gab1 and anti-phosphotyrosine antibodies. Therefore, immune pellets were washed twice with each of the following buffers: 1) PBS containing 1% Nonidet P-40 and 200 μM Na3VO4, 2) 100 mM Tris, pH 7.4, 0.5 mM LiCl, and 200 μM Na3VO4 and 3) 10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, and 200 μM Na3VO4. The PI 3-kinase assay was performed directly on the immune pellets as described previously (24). Briefly, 10 μg of sonicated 1,2-phosphatidylinositol in 10 mM HEPES, 1 mM EGTA, pH 7.5, were added to the immune pellets, and the reaction was started by the addition of 25 μl of a reaction mixture consisting of 40 mM HEPES, pH 7.4, 20 mM MgCl2, 80 μM ATP, and 5 μCi of [γ-32P]ATP. After 20 min the reaction was stopped by the addition of 40 μl of 4 N HCl and 160 μl of CHCl3/CH3OH/H2O/NH4OH (60:47:8.8:4.4) solvent system, phosphatidylinositol phosphate was visualized by autoradiography. Preparation of Plasma Membrane Lawns, Immunofluorescence, and Image Analysis—The 3T3-L1 cells were grown on glass coverslips and differentiated as described above. After serum starvation the 3T3-L1 adipocytes were washed with Krebs-Ringer phosphate buffer and incubated for 10 min in Krebs-Ringer phosphate buffer supplemented with 0.2% bovine serum albumin and 1.25 mM CaCl2. Cells were then either left untreated or stimulated with HGF (100 ng/ml) or insulin (0.5 nm) for 20 min. Plasma membrane lawns were prepared as previously described (25). Briefly, after 2 washes with ice-cold PBS, cells were fixed with 0.55 mg/ml poly-L-lysine for 1 min at 4 °C and then swollen by three successive rinses with a hypotonic buffer (30 mM HEPES, pH 7.5, 70 mM KCl, 5 mM MgCl2, 3 mM EGTA). The swollen cells were sonicated in the hypotonic buffer supplemented with 1 mM dithiothreitol and proteases inhibitors, and the bound membrane sheets were fixed with 4% paraformaldehyde and blocked with PBS containing 1% bovine serum albumin and 4% calf serum. Thereafter, plasma membrane lawns were incubated with anti-Glut1 or anti-Glut4 antibodies (5 μg/ml in blocking buffer) for 1 h at room temperature and washed 3 times 10 min with blocking buffer. They were then incubated for 1 h at room temperature with fluorescein isothiocyanate conjugated secondary antibodies and wheat germ agglutinin-Texas Red to normalize. After three 10-min washes with blocking buffer, the coverslips were mounted in Mowiol onto glass slides. The plasma membrane lawns were analyzed with an Axiovert 200 microscope using a Plan-Neofluar 40 × 1.3 numerical aperture oil objective (Carl Zeiss, Göttingen, Germany). Images were acquired using a cooled digital camera (CoolSnap HQ, Roper Scientific Princeton Instruments, Evry, France), and quantification was made using Metamorph image analysis software with auto-threshold detection of pixels (Universal Imaging Corp., Downingtown, PA) as described previously (25).

Real-time Quantitative PCR Analysis—Total RNAs from 3T3-L1 cells and mouse white adipose tissue were isolated using the RNaBle total RNA extraction kit (Eurobio, France) and TRIzol reagent (Invitrogen), respectively. cDNA was synthesized using the Standard Two-step RT-PCR-μGO™ kit (MP Biomedicals Europe) from 1 μg of total RNA in a final volume of 100 μl. Real time quantitative PCR was performed in duplicate with the ABI PRISM 7000 sequence detection system and SYBRGreen dye (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocol. Primers were designed using Primer Express program (Applied Biosystems). The list of sequences used for primers is available upon request (gual@unice.fr). The reaction mix consisted of 12.5 μl of qPCR MasterMix Plus for SYBR® green I (Eurogentec, Seraing, Belgium), 400 nM forward and reverse primers, and 5 μl of 1/10 cDNA in a final volume of 25 μl. PCR conditions were as follows: 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Amplification of specific transcripts was confirmed by melting curve profiles generated at the end of the PCR program. The mRNA levels of genes of interest (R) were normalized to mRNA levels of 36B4: \[ \Delta Ct_R = Ct_R - Ct_{36B4} \] The relative amount of interest mRNA between the 3T3-L1 cells or two groups of mice is given by \[ 2^{-\Delta \Delta Ct} \], where \[ \Delta \Delta Ct = (\Delta Ct_{R \text{ of untreated cells}}) - (\Delta Ct_{R \text{ of treated cells}}) \] or \[ \Delta \Delta Ct = (\Delta Ct_R \text{ of obese mice}) - (\text{mean of } \Delta Ct_R \text{ of lean mice}) \].

Calculation and Statistical Analysis—Data are expressed as the means ± S.E. The statistical difference between mRNA levels in obese and lean mice was determined with the non-parametric Kruskal-Wallis test using the ΔΔCt of each group. Other data were statistically analyzed using Student’s t test. p < 0.05 was considered significant.

RESULTS

The Gene Expression of HGF Was Increased in Adipose Tissue from Obese Diabetic Mice—We first examined whether HGF and HGF receptor were differentially expressed in white adipose tissue from lean and obese diabetic mice. Total RNAs were isolated from the epididymal fat pads of lean db/+ mice and obese diabetic db/db mice, and the gene expression levels of HGF and HGF receptor were determined using real-time quantitative PCR. As shown in Fig. 1A, the HGF gene expression was significantly increased in adipose tissue from obese diabetic db/db mice compared with lean controls, whereas the gene expressions of HGF receptor were unchanged. We then evaluated the gene expression levels of HGF and its receptor in 3T3-L1 fibroblasts and 3T3-L1 adipocytes. As shown in Fig. 1B, HGF was strongly expressed in 3T3-L1 fibroblasts, and its receptor was much more expressed in 3T3-L1 adipocytes. To secure the quality of the cell differentiation, aP2 was used as a marker of adipocyte differentiation and was found to be predominantly expressed in 3T3-L1 adipocytes (data not shown).

HGF Stimulated Glucose Uptake in 3T3-L1 Adipocytes—We then examined whether HGF was able to induce glucose transport in 3T3-L1 adipocytes. Cells were incubated with different concentrations of HGF (from 12.5 to 200 nm) for 20 min, and deoxyglucose uptake was measured. HGF treatment resulted in a significant increase in deoxyglucose uptake in a dose-dependent manner with a maximal effect at 100 ng/ml (~3-fold increase over control).
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FIGURE 1. HGF mRNA expression was increased in adipose tissue from obese diabetic db/db mice compared with lean controls. A, total RNAs were isolated from epididymal adipose tissue of obese diabetic db/db mice (n = 6) and their lean control littermates (n = 6) as described under “Experimental Procedures.” HGF and HGF receptor (HGF-R) mRNA levels were analyzed by real-time quantitative PCR, normalized to mRNA levels of 36B4, and expressed relative to the expression levels in fibroblasts. B, total RNAs were isolated from 3T3-L1 fibroblasts and 3T3-L1 adipocytes as described under “Experimental Procedures.” HGF and HGF receptor mRNA levels were analyzed by real-time quantitative PCR, normalized to mRNA levels of 36B4, and expressed relative to the expression levels in fibroblasts. The means ± S.E. of six independent experiments are shown. *, p < 0.05.

FIGURE 2. HGF dose-dependently stimulated glucose transport in 3T3-L1 adipocytes. After serum starvation, 3T3-L1 adipocytes were incubated or not with different concentrations of HGF as indicated for 20 min. Uptake of 2-[3H]deoxyglucose (2-DOG) was then measured during a 3-min period as described under “Experimental Procedures.” The means ± S.E. of three independent experiments are shown. In the same conditions 0.5 nM insulin induced a 6-fold, and 100 nM insulin induced a 12-fold increase in 2-[3H]deoxyglucose uptake.

increase) (Fig. 2). By comparison, 0.5 nM insulin induced a 6-fold, and 100 nM insulin induced a 12-fold increase in glucose uptake (data not shown).

HGF Enhanced Glut4 Translocation in 3T3-L1 Adipocytes—Glucose transport is dependent on the translocation of specific glucose transporters, including Glut1 and Glut4, from intracellular compartments to the plasma membrane (3). To determine the effect of HGF on the translocation of both Gluts, plasma membrane lawns were prepared from 3T3-L1 adipocytes incubated with or without HGF (100 ng/ml) or insulin (0.5 nM) for 20 min, and the amount of plasma membrane-associated glucose transporters was analyzed by immunofluorescence with specific antibodies. As previously described (3, 5), insulin stimulation was mainly associated with the translocation to the plasma membrane of Glut4. Whereas insulin at 0.5 nM enhanced the translocation of Glut4 but not Glut1 (Fig. 3), a supraphysiological concentration of insulin (100 nM) stimulated the translocation of both glucose transporters (data not shown). HGF also significantly increased (∼3-fold) Glut4 translocation to the plasma membrane without any effect on Glut1 (Fig. 3).

Activation of PI 3-Kinase Was Required for HGF-induced Glucose Uptake in the 3T3-L1 Adipocytes—It is well established that activation of PI 3-kinase is necessary for insulin-stimulated Glut4 translocation and glucose transport (3, 5, 26). We, therefore, tested whether PI 3-kinase activation was also required for HGF effect. 3T3-L1 adipocytes were pretreated with a PI 3-kinase inhibitor (wortmannin, 100 nM) before HGF stimulation (100 ng/ml), and deoxyglucose uptake was measured. We observed that inhibition of the PI 3-kinase activity by wortmannin abolished HGF-induced glucose uptake (Fig. 4A), indicating that glucose uptake in response to HGF is completely dependent on the PI 3-kinase pathway. Among the PI 3-kinase effectors, PKB and its substrate AS160 regulate the insulin-stimulated Glut4 translocation in adipocytes (4). To investigate whether HGF also promoted PKB and AS160 phosphorylation, 3T3-L1 adipocytes were incubated with or without HGF (100 ng/ml) or insulin (0.5 nM) for 10 min, and the phosphorylation levels of PKB and AS160 were analyzed by Western blotting with anti-phosphospecific antibodies. As shown in Fig. 4B, HGF, like insulin, induced the phosphorylation of PKB and AS160. Thus, activation of the PI 3-kinase/PKB/AS160 pathway played an important role in the HGF-stimulated glucose transport.

HGF Stimulated the PI-3 Kinase Activity Mainly via Gab1 in 3T3-L1 Adipocytes—It has been reported in several cell lines that the majority of the HGF-stimulated PI 3-kinase activity is associated with the docking protein Gab1 (20, 22). In contrast, the insulin-stimulated PI 3-kinase activity is mainly associated with IRS1, although the insulin receptor can also phosphorylate Gab1 in fibroblasts and hepatocytes (27, 28). We, therefore, attempted to assess the relative contribution of Gab1 and IRS1 to recruit the p85 subunit of the PI 3-kinase upon HGF or insu-
lin stimulation. Gab1 and IRS1 were immunoprecipitated from 3T3-L1 adipocytes treated or not with HGF (100 ng/ml) or insulin (0.5 nM) for 10 and 20 min. Their tyrosine phosphorylation levels and the association of p85 were analyzed by Western blotting with anti-phosphotyrosine and anti-p85 antibodies, respectively. As seen in Fig. 5, A and B, HGF treatment led to a large increase in the tyrosine phosphorylation of Gab1 and the recruitment of the p85 subunit of PI 3-kinase. In contrast, in response to insulin, Gab1 was weakly tyrosine-phosphorylated and did not recruit the p85 subunit of PI 3-kinase. Whereas no phosphorylation of IRS1 was observed upon HGF stimulation, insulin elicited, as expected, the tyrosine phosphorylation of IRS1 leading to the recruitment of the p85 subunit of PI 3-kinase (Fig. 5A). Taken together these findings indicated that the Gab1/PI 3-kinase complex was stimulated in response to HGF, whereas the IRS1/PI 3-kinase complex was triggered upon insulin stimulation. Because PI 3-kinase could be directly recruited by the HGF receptor, we therefore assessed the relative contribution of Gab1 in the HGF-stimulated PI 3-kinase activity. 3T3-L1 adipocytes were incubated with HGF (100 ng/ml) for 10 min. Gab1 and tyrosine-phosphorylated proteins were successively immunoprecipitated from the total lysates, and the PI 3-kinase activity associated to the immune pellets was determined. Although HGF induced a ~4-fold increase in PI 3-kinase activity associated to Gab1, a low amount of activity remained associated to tyrosine-phosphorylated proteins after Gab1 immunoprecipitation (Fig. 6). This indicates that most of the PI 3-kinase activity was associated to Gab1 upon HGF stimulation.

Chronic TNFα Treatment of 3T3-L1 Adipocytes Induced an HGF Resistance State—Previous studies have shown that prolonged TNFα treatment of 3T3-L1 adipocytes causes a state of insulin resistance leading to a strong decrease in IRS1 protein levels, insulin-induced PKB activation, and glucose uptake (29). We, therefore, determined whether HGF could still mediate its effect in insulin resistant adipocytes. Cells were pretreated with TNFα (50 ng/ml) for 24 h before stimulation with HGF (100 ng/ml) or insulin (0.5 nM) for 20 min, and deoxyglucose uptake was then measured. Although TNFα treatment induced a significant increase in the basal glucose transport, the HGF and insulin effects on glucose transport were markedly reduced (33 ± 10 and 62 ± 6% of inhibition, respectively) (Fig. 7A). The impairment of HGF effect was correlated with a strong decrease in Gab1 protein levels and HGF-stimulated PKB phosphorylation (Fig. 7B), indicating that adipocytes rendered insulin resistant by TNFα lost their sensitivity to HGF.

Rosiglitazone Treatment Increased HGF and HGF Receptor Gene Expression in 3T3-L1 Adipocytes—Because thiazolidinedione, an anti-diabetic drug, decreased the expression of TNFα in adipose tissue, prevented its inhibitory effects in
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3T3-L1 adipocyte (29, 30), and increased the expression of HGF receptor in hepatocytes (31), we investigated its effect on the expression of HGF and HGF receptor in 3T3-L1 adipocytes. As shown in Fig. 8, the expression levels of HGF and its receptor were 3-fold increased after the treatment of 3T3-L1 adipocytes with rosiglitazone (100 nM) for 48 h. This suggested that the beneficial effects of thiazolidinedione (TZD) on glucose homeostasis could be associated not only with the improvement of insulin signaling but also with the enhancement of HGF signaling.

DISCUSSION

In the present study we have shown that the gene expression of HGF is strongly increased in adipose tissue from obese diabetic mice compared with lean mice. In 3T3-L1 cells, HGF was predominantly expressed in fibroblasts compared with adipocytes, and HGF enhanced glucose uptake and lipogenesis (data not shown) in adipocytes. These results could suggest that the local production of HGF, mainly secreted by the fibroblasts, could regulate the functions of the adipocytes. In favor of this, it has been reported that a large part of the HGF release (>90%) could be attributed to the nonfat cells (11) such as preadipocytes, fibroblasts, macrophages, and endothelial cells (11, 32–35). However, HGF was also expressed to a lesser extent by 3T3-L1 adipocytes, suggesting an autocrine loop in adipocytes in addition to paracrine effects from HGF produced in stromal cells.

After secretion, HGF has to be cleaved to be active (36). It seems to be the case since HGF secreted by the adipose tissue stimulated the growth of a HGF receptor-expressing mouse mammary carcinoma (10) and promoted tube formation of vascular endothelial cells in vitro (37). HGF belongs to the adipokines that are present in blood at very low concentrations (0.7 ng/ml in lean patients, 2.5 ng/ml in morbidly obese patients, 5 ng/ml in mice) (13). However, in adipose tissue explants a large
secretion of HGF occurs compared with serum concentration (11). Furthermore, since HGF could be retained with extracellular matrix in the producing tissue, its local concentration could be much greater than circulating levels. Thus, although the circulating level of HGF is low, the large production of active HGF by adipose tissue could have a local role.

HGF and insulin stimulated glucose uptake in 3T3-L1 adipocytes via a mechanism exclusively or mainly dependent on the PI 3-kinase activity, respectively. However, insulin and HGF activated the PI 3-kinase through different docking proteins; IRS1 in response to insulin and Gab1 upon HGF stimulation. Whereas insulin weakly stimulated the phosphorylation of Gab1, we did not detect the recruitment of the p85 subunit of PI 3-kinase. In agreement with this, it has been reported that the insulin-induced PI 3-kinase activity was mainly associated with IRS1 in 3T3-L1 adipocytes. Interestingly, a large part of this activity was associated with Gab1 in undifferentiated fibroblasts (27). This suggests a different role of Gab1 in insulin signaling depending on cell type.

The role of Gab1 in glucose uptake has already been reported. The microinjection of anti-Gab-1 antibodies strongly inhibits osmotic shock-induced Glut 4 translocation without altering insulin effect (38). We have previously shown that osmotic shock-induced tyrosine phosphorylation of Gab1 led to glucose uptake via a mechanism independent of the PI 3-kinase activity (23). On the contrary, the phosphorylation of Gab1 in response to HGF mainly activated the PI 3-kinase/PKB/AS160 pathway, leading to the subsequent glucose uptake. This indicates that HGF could activate different signaling pathways dependent on Gab1, but activation of the PI 3-kinase played a critical role in HGF-stimulated glucose transport. Therefore, the activation of Gab1 dependent pathways could be a good target to enhance glucose uptake in adipocytes in insulin resistance and diabetes. It was indeed not the case since the HGF signaling pathway was also altered in adipocytes treated with TNFα. In these insulin-resistant adipocytes, the amount of Gab1 was decreased and correlated with an impairment of the HGF-stimulated PKB activation and glucose uptake.

The partial correction of the insulin resistance has been reported after the treatment with TZD, an anti-inflammatory and anti-diabetic drug. TZD inhibits the secretion of cytokines such as TNFα by the adipose tissue and prevents its inhibitory effect in vitro (29, 30). It is interesting to note that we have determined that TZD also enhanced the gene expression of both HGF and HGF receptor in 3T3-L1 adipocytes. In addition, Saiki et al. (37) have recently reported the increase in HGF protein expression after TZD stimulation. In vivo the treatment of rats with TZD increased hepatic HGF receptor expression and induced its tyrosine phosphorylation (31). Furthermore, treatment of type 2 diabetic patients with TZD increased the plasma HGF levels (37). Whereas further studies are required to determine the direct link between activation of HGF signaling and improvement of metabolic parameters, the beneficial effects of TZD on glucose homeostasis could be associated not only with the improvement of insulin signaling but also with the enhancement of HGF signaling.

The secretion of HGF by adipose tissue could, therefore, contribute to elevated serum HGF in obesity, whereas its role in obesity has not yet been identified. We have shown here that the production of HGF by adipose tissue could play a local role because it stimulated glucose uptake in adipocytes. The insulin mimetic effect of HGF is dependent on different early proximal signaling events from those activated by insulin. However, the role of local production of HGF in adipose tissue growth should also be investigated in a future issue.

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