The ability of the growth factors epidermal growth factor (EGF), transforming growth factor α (TGFα), and platelet-derived growth factor to exert insulin-like effects on glucose transport and lipolysis were examined in human and rat fat cells. No effects were found in rat fat cells, whereas EGF (EC₅₀ for glucose transport ~0.02 nM) and transforming growth factor α (EC₅₀ ~0.2 nM), but not platelet-derived growth factor, mimicked the effects of insulin (EC₅₀ ~0.2 nM) on both pathways. EGF receptors, but not TGFα, were abundantly expressed in human fat cells as well as in human skeletal muscle. EGF increased the tyrosine phosphorylation of several proteins (the EGF receptor, insulin receptor substrate (IRS)-1, IRS-2, and Grb2-associated binder 1), whereas Shc and Gab2 were only weakly and inconsistently phosphorylated. p85, the regulatory subunit of phosphatidylinositol 3-kinase (PI 3-kinase), was also found to associate with all of these docking molecules, showing that EGF activated PI 3-kinase pools that were additional to those of insulin. EGF and/or insulin increased protein kinase B/Akt serine phosphorylation to a similar extent, whereas mitogen-activated protein kinase phosphorylation was more pronounced for EGF than for insulin. The impaired insulin-stimulated downstream signaling, measured as protein kinase B/Akt serine phosphorylation in insulin-resistant cells (Type 2 diabetes) was improved by the addition of EGF. Thus, EGF receptors, but not TGFα, are abundantly expressed in human fat cells and skeletal muscle. EGF mimics the effects of insulin on both the metabolic and mitogenic pathways but utilize in part different signaling pathways. Both insulin and EGF increase the tyrosine phosphorylation and activation of IRS-1 and IRS-2, whereas EGF is also capable of activating additional PI 3-kinase pools and, thus, can augment the downstream signaling of insulin in insulin-resistant states like Type 2 diabetes.
cytoplasmic IRS molecules, which can be targeted to the intracellular microsomal and GLUT4-containing compartment (4, 16). Grb2-associated binder 1 (Gab1) and the closely related protein, Gab2 (17, 18), belong to the IRS-like family of proteins. Gab1 and Gab2 have been shown to be important mediators of the signal transduction of growth factors and cytokines (18, 19). Gab1 becomes tyrosine-phosphorylated by insulin, EGF, and other growth factors in A431 cells (20) and can bind the p85 subunit and activate PI 3-kinase in response to EGF in different cell lines (20). In contrast, in a recent study, Gab2 was found to be the major target for PI 3-kinase activation by EGF in rat hepatocytes (21).

In the present study, we examined the metabolic effect of different growth factors in human and rat fat cells. We found that EGF, in contrast to other growth factors, exerted a full insulin-like effect in human, but not rat, fat cells. This species difference is due to the very abundant expression of EGFR in human, but not rat, adipocytes. We also compared the intracellular signaling pathways for insulin, EGF and TGF-α: the latter hormone is also a ligand for the EGFR. EGF/TGF-α increase the tyrosine phosphorylation and p85 binding to several intracellular docking proteins (IRS-1, IRS-2, Gab1, and EGFR), leading to the activation of both the metabolic and mitogenic pathways. Furthermore, by activating PI 3-kinase pools that are additional to those of insulin, EGF can also augment the downstream signaling of insulin in insulin-resistan states like Type 2 diabetes.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human insulin was from Novo Nordisk (Copenhagen, Denmark). AG1478 and LY294002 were from Calbiochem, and bovine serum albumin (fraction V), collagenase,wortmannin, and other fine chemicals were from Sigma. Radiochemicals were from Amersham Biosciences. Anti-IRS-1, anti-IRS-2, anti-PI 3-kinase, anti-Shc, anti-Gab1, and anti-Gab2 antibodies were purchased from Upstate Biotechnology Inc. (Lake Placid, NY). Protein A/G Plus-agarose and anti-phosphotyrosine monoclonal antibodies (Tyr(P)E7) were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), anti-phospho-MAP kinase antibodies were from Promega (Madison, WI), and anti-phospho-serine (Ser473) PKB/Akt antibodies were from Biolab (Boston, MA). GLUT-4 antibodies were a kind gift from Dr. Samuel Cushman (NIDDK, National Institutes of Health).

**Patients and Source of Adipose Tissue and Skeletal Muscle**—Specimens of human subcutaneous adipose tissue were obtained from the abdominal region of subjects undergoing elective surgery for nonmalignant disease. Humans, who had been fed a liquid diet for at least 24 h, were anesthetized by injection of sodium pentobarbital (12 mg/kg) intraperitoneally. The abdominal fat was removed, and the adipocytes were isolated by differential centrifugation as previously described (23). Cells were then incubated with the various additions as indicated.

**Preparation of Adipose Cells and Immunoprecipitations**—Isolated adipocytes were from human, but not rat, adipocytes. We also compared the intracellular signaling pathways for insulin, EGF and TGF-α: the latter hormone is also a ligand for the EGFR. EGF/TGF-α increase the tyrosine phosphorylation and p85 binding to several intracellular docking proteins (IRS-1, IRS-2, Gab1, and EGFR), leading to the activation of both the metabolic and mitogenic pathways. Furthermore, by activating PI 3-kinase pools that are additional to those of insulin, EGF can also augment the downstream signaling of insulin in insulin-resistant states like Type 2 diabetes.

**Preparation of Adipose Cells and Plasma Membranes**—Adipose cells were prepared according to methods previously described (22, 23). The tissue was cut into small fragments visibly free of connective tissue and clotted blood. About 0.6 g of tissue was incubated at 37 °C in Medium 199 Hanks containing 4% bovine serum albumin, 5.5 mM glucose, and 0.8 mg/ml collagenase in a shaking water bath. After ~50 min, isolated cells were filtered through a nylon mesh (pore size, 400 μm) and washed four times in fresh bovine serum albumin-containing medium before final resuspension at 2% cytocr. Cell size and number were measured as previously described (23). Cells were then incubated with the various additions as indicated.

Subcellular fractions enriched in plasma membranes were isolated following homogenization and differential centrifugation as described previously (22, 24). The membranes were resuspended in TES (20 mM Tris-HCl, 1 mM EDTA, 0.25 mM sucrose) and assayed for proteins by the bicinchoninic acid method using a commercial kit (Pierce). Aliquots (30 μg of protein) were mixed with same volume of sample buffer, loaded on SDS-PAGE 10% and then electrophoretically transferred onto nitrocellulose paper. Immunoblotting was performed as described below.

Glucose transport activity was assayed for 1 h with 0.86 μM [U-14C]glucose as previously described. After 1 h, the cells were separated from the incubation medium by centrifugation through silicone oil, and the radioactivity associated with the cells was measured by scintillation counting (22).

**Preparation of Adipose Cells and Immunoprecipitations**—Isolated human adipocytes were distributed into plastic vials (12–15% cell suspension) in a final incubation volume of 500 μl. Cells were preincubated with the indicated agents for 15 min, immediately separated by centrifugation through silicone oil and suspended in 0.4 ml of lysis buffer containing 25 mM Tris-HCl, pH 7.4, 0.5 EGTA, 25 mM NaCl, 1% Nonidet P-40, 1 mM Na3VO4, 10 mM NaF, 0.2 mM leupeptin, 1 mM benzamidine, 0.1 mM (6-aminohexyl)-benzene-sulfonfyl fluoride, and 0.1 mM o-phenanthroline acid and rocked for 2 h at 4 °C. Detergent-insoluble material was sedimented by centrifugation at 12,000 × g for 5 min at 4 °C, and the supernatants were immunoprecipitated with the different antibodies according to the recommendations of the manufacturers. Subsequently, the immune complexes were precipitated with Protein A/G Plus-agarose for 90 min at 4 °C. The immunoprecipitates were washed twice with lysis buffer.

**Immunoblotting**—Crude cell extracts or specific immunoprecipitations were boiled in Laemmli buffer containing 150 mM diethiothreitol for 5 min. The samples were analyzed by SDS-polyacrylamide gel electrophoresis (6, 7, 5, 8%). Proteins were transferred from the gel to nitrocellulose sheets and blocked in 5% fat-free milk. The blots were probed with the different primary antibodies according to the recommendations of the manufacturers. The proteins were detected by enhanced chemiluminescence using horseradish peroxidase-labeled secondary antibodies (Amersham Biosciences), and the intensity of the bands was quantitated with a laser densitometer (Amersham Biosciences).

**Phosphatidylinositol 3-Kinase Activity**—The PI 3-kinase assay was performed directly on IRS-1 or Gab1 immunoprecipitates as previously described (22). Briefly, after the washings described above, 6 μl of a mixture of phosphatidylinositol (10 μg/sample) and phosphatidylinerine (2.5 μg/sample) were added to the beads. The reaction was started by the addition of 30 μl of a reaction mixture containing 40 mMol/liter Hepes, pH 7.5, 20 μmol/liter MgCl2, 50 μmol/liter [32P]ATP (0.2 μCi/μl). The reaction was stopped by the addition of 40 μl of HCl (4 N) and 160 μl of CHCl3/CH3OH/CH3COOH (3:1:1). The organic phase was extracted and applied to a silica gel thin layer chromatography plate precoted with 1% potassium oxalate (Analtech, Newark, DE). The thin layer chromatography plates were developed in CHCl3/CH3OH/H2O/NH4OH (60:47:11.3:2), dried, and visualized by autoradiography. The radioactivity was quantitated with a PhosphorImager (Amersham Biosciences).

**Real Time RT-PCR**—Real time RT-PCR was used to examine the gene expression for EGF and TGF-α in fat cells and for EGFR in both fat cells and skeletal muscle from the same individuals. RNA was extracted, and mRNA levels for specific gene sequences were measured as previously reported in detail (25, 26). The sequences used for the primers and probes were as follows: EGFR forward, 5’-GATATTTAAGGACTT; EGFR reverse, 5’-GATACCTGCTCCCAGTATG; EGFR probe, 5’-FAM-AACAAGTATGACTAAGCTGTTGTTGACACACCAAC-TAMRA-3’; TGF-α forward, 5’-GGCAGGATGTTGCTGCTGACT; TGF-α reverse, 5’-CCCCACCAAGGGCTTGA; TGF-α probe, 5’-FAM-CTTCTCTGTCGTCGGACACACACAC-TAMRA-3’; EGFR forward, 5’-CCCTGGCCAGCTGGCAACA; EGFR reverse, 5’-GCCACAGATGTATTTGCTGATT; EGFR probe, 5’-FAM-CTTCTCTGTCGTCGGACACACACAC-TAMRA-3’.

**RESULTS**

**Insulin-like Effects of EGF/TGF-α**—High concentrations of EGF/TGF-α, but not PDGF, increased glucose transport to the same extent as insulin, and no additive effect was seen when both EGF and insulin were present (Fig. 1A). Like insulin, glucose transport was only seen in response to EGF in adipocytes treated by both wortmannin and ML-9, inhibitors of PI 3-kinase (27) and PDK/ Akt (8), respectively. Furthermore, the increased glucose transport induced by both hormones was associated with the translocation of GLUT4 from an intracellular microsomal compartment to the plasma membrane (Fig. 1A). Although the rates of glucose transport and translocation from the microsomal fractions were the same, insulin tended to lead to a greater recovery of GLUT4 in the plasma membrane, a finding that was reproduced in three independent experiments.
TGF-α, which also binds to EGFR (28, 29), also increased glucose transport to the same extent as insulin and with a similar affinity (EC₅₀ ~0.2 nm) (Fig. 1B and data not shown). In contrast, EC₅₀ for EGF (~0.02 nm) was considerably lower than for either insulin or TGF-α (Fig. 1C).

EGF also exerted a similar antilipolytic effect as insulin, and both effects were inhibited by wortmannin (Fig. 1D). Taken together, these findings show that EGF inhibits lipolysis and stimulates glucose transport to the same extent as insulin but with a considerably lower EC₅₀. Furthermore, the effects of both hormones were inhibited by wortmannin as well as another inhibitor LY294002 (data not shown), suggesting that they were mediated through PI 3-kinase activation.

**EGF- and/or Insulin-stimulated Tyrosine Phosphorylation**

As shown in Fig. 2A, EGF increased the tyrosine phosphorylation of major bands at ~100 and 185–200 kDa, whereas the major insulin-stimulated phosphorylations were seen at ~97 (insulin receptor) and 185 kDa (IRS). The EGF-stimulated tyrosine-phosphorylated ~100-kDa band was identified as Gab1 by both co-localization (Fig. 2A) and immunoprecipitation, which also precipitated activated EGFR (Fig. 2B). Insulin did not increase the tyrosine phosphorylation of Gab1 in human fat cells. Gab1 tyrosine phosphorylation by EGF was also clearly inhibited by wortmannin, suggesting that it was mediated through PI 3-kinase activation (Fig. 2B, bottom). The 185–200-kDa bands phosphorylated by EGF were identified as both the EGFR and IRS-1 and IRS-2 (Fig. 2C). The ability of EGF to increase tyrosine phosphorylation of IRS-1 was rapid and was seen within 2 min (data not shown).

To further explore the possibility that the activated EGFR can bind to and phosphorylate IRS-1, co-immunoprecipitation experiments were performed. EGFR was recovered in IRS-1 immunoprecipitates (Fig. 2C), and, similarly, EGF increased the recovery of IRS-1 in EGFR immunoprecipitates (data not shown).

Although EGF exerted insulin-like effects on glucose transport and lipolysis and increased the tyrosine phosphorylation of both IRS-1 and IRS-2, this was not due to activation of the insulin receptor, since EGF, in contrast to insulin, did not increase the tyrosine phosphorylation of the insulin receptor (Fig. 2D).

Taken together, these findings show that both EGF and insulin increase the tyrosine phosphorylation of IRS-1 and IRS-2, while still showing specificity for their cognate receptors, and, in addition, EGF increases tyrosine phosphorylation of Gab1 through a wortmannin-inhibitable and, thus, presumably PI 3-kinase-dependent pathway.

The ability of EGF, but not insulin, to phosphorylate and activate Gab1 is also consistent with the finding that Gab1 was shown. Results are means ± S.E. of eight separate experiments. Bottom, effect of insulin and EGF on the translocation of GLUT 4 from the plasma membrane (PM) to the microsomal fraction (LDM). Isolated cells were incubated with 6.9 nm insulin or 50 nm EGF for 30 min, and subcellular fractions were prepared as described under “Experimental Procedures.” Results are from one representative experiment repeated three times. B, the effect of different concentrations of TGF-α and insulin 6.9 nm on glucose transport in human adipocytes. The glucose transport assay was performed as described under “Experimental Procedures” and shows one representative experiment repeated twice. C, the effect of different concentrations of EGF and insulin 6.9 nm on glucose transport in human adipocytes. The glucose transport assay was performed as described under “Experimental Procedures” and shows one representative experiment repeated twice. D, insulin and EGF decreased 8-bromo-cyclic AMP (8-Br-cAMP)-stimulated lipolysis. Isolated adipocytes were incubated with or without 4 μM 8-bromo-cyclic AMP with the addition of 6.9 nm insulin or 50 nm EGF (upper panel) and the glyceral release measured. The addition of 1 μM wortmannin inhibited the effect of both insulin and EGF (lower panel).

**Fig. 1.** A, top panel, glucose transport was assessed in isolated human adipocytes incubated with no addition (bas), 6.9 nm insulin (ins), 50 nm EGF alone or in combination with insulin and PDGF alone (10 nm). The effect of 1 μM wortmannin (Wort) and 300 μM ML9 is also shown. Results are means ± S.E. of eight separate experiments. Bottom, effect of insulin and EGF on the translocation of GLUT 4 from the plasma membrane (PM) to the microsomal fraction (LDM). Isolated cells were incubated with 6.9 nm insulin or 50 nm EGF for 30 min, and subcellular fractions were prepared as described under “Experimental Procedures.” Results are from one representative experiment repeated three times. B, the effect of different concentrations of TGF-α and insulin 6.9 nm on glucose transport in human adipocytes. The glucose transport assay was performed as described under “Experimental Procedures” and shows one representative experiment repeated twice. C, the effect of different concentrations of EGF and insulin 6.9 nm on glucose transport in human adipocytes. The glucose transport assay was performed as described under “Experimental Procedures” and shows one representative experiment repeated twice. D, insulin and EGF decreased 8-bromo-cyclic AMP (8-Br-cAMP)-stimulated lipolysis. Isolated adipocytes were incubated with or without 4 μM 8-bromo-cyclic AMP with the addition of 6.9 nm insulin or 50 nm EGF (upper panel) and the glyceral release measured. The addition of 1 μM wortmannin inhibited the effect of both insulin and EGF (lower panel).
translocated to the plasma membrane in response to EGF but not insulin (Fig. 2D). We also examined whether EGF increased the tyrosine phosphorylation of Gab2 or Shc. However, no clear and consistent effect on either of these proteins was found (data not shown).

The ability of insulin and EGF to increase tyrosine phosphorylation in rodent fat cells, where EGF did not increase either glucose transport or inhibit lipolysis (data not shown) in agreement with previous findings (13), was also examined. In contrast to human fat cells, neither Gab1 nor IRS-1 or IRS-2 were
phosphorylated by EGF (Fig. 2E). The weak tyrosine-phosphorylated band at the size of EGFR suggests that rat fat cells have very few EGFR, as also previously reported (13).

Association with p85 and PI 3-Kinase Activity—We next examined whether the p85 regulatory subunit of PI 3-kinase associated with tyrosine-phosphorylated EGFR, IRS-1, and Gab1 in response to EGF. As shown in Fig. 3, EGF increased the association of p85 with all of these docking proteins without any additional effect by the presence of insulin, whereas insulin alone only increased the association with IRS-1. Similarly, both insulin and EGF increased the p85 binding to IRS-2 (data not shown). We also measured PI 3-kinase activity in response to EGF in IRS-1 and Gab1 immunoprecipitates. EGF increased PI 3-kinase activity associated with IRS-1 ~100%, whereas the increase in Gab1-associated activity was more marked (4–500%) (data not shown).

Activation of PKB/Akt and MAP Kinase—As a measure of downstream activation, we examined the ability of EGF and/or insulin to increase the threonine and serine phosphorylation of PKB/Akt. As shown in Fig. 4, both EGF and insulin increased the phosphorylation of PKB/Akt to a similar extent. In contrast, tyrosine/threonine phosphorylation of MAP kinase was more pronounced in the fat cells following incubation with EGF than with insulin (Fig. 4).

EGF Augments Downstream Insulin Signaling in Insulin Resistance—The studies above show that EGF activates PI 3-kinase in pools (IRS-1, IRS-2, Gab1, and EGFR) that are additional to those of insulin (IRS-1/2).

We then studied whether EGF can be used to augment insulin action in insulin-resistant states by characterizing the ability of insulin alone or in combination with EGF to increase the downstream insulin signaling, measured as serine phosphorylation of PKB/Akt, in fat cells from eight Type 2 diabetic subjects. As discussed above and shown in Fig. 4, EGF increases Ser(P)-PKB/Akt to a similar extent to insulin in normal (noninsulin-resistant) individuals, and no additive effects were found.

However, insulin-stimulated Ser(P)-PKB/Akt is markedly reduced in cells from eight Type 2 diabetic subjects (30, 31) where IRS-1 protein expression is reduced (32). Fig. 5 shows that the insulin-stimulated tyrosine phosphorylation of IRS was reduced in diabetic cells, whereas the ability of EGF alone or in combination with insulin to increase the phosphorylation of Gab1 was normal. This is consistent with the finding that Gab1 protein expression was similar in control and diabetic cells (data not shown).

DISCUSSION

In this paper, we show for the first time that the growth factors EGF/TGF-α, but not PDGF, exert full insulin-like effects in human fat cells and that EGF requires a much lower concentration than insulin to elicit these effects (EC_{50} ~0.02 nm versus 0.2 nm). These findings, together with the high mRNA expression of EGF, the marked receptor tyrosine phosphorylation by EGF as well as an abundant EGFR protein expression clearly document that EGF receptors are very highly expressed in human fat cells. This is markedly different from rat fat cells, which have a very low expression of EGF receptors as also previously reported (13). This difference is probably the major reason for the finding that EGF does not exert any insulin-like effects in rat fat cells. In contrast, EGF...
receptors appear to be more abundantly expressed in mouse fat cells, including 3T3-L1 cells (33), where a partial insulin-like effect has been reported (34). In general, growth factors do not exert insulin-like effects despite the fact that they increase PI 3-kinase activity. This has been attributed to an inability to activate PI 3-kinase in appropriate subcellular compartments (16, 35), to differential activation of PKB isoforms (9, 36), and/or to an increased serine and reduced tyrosine phosphorylation of IRS-1 (37). However, the present data clearly show that some growth factors, like EGF/TGF-α, can exert full insulin-like effects if the cognate receptors are abundantly expressed together with the downstream signaling molecules.

The EGF receptor has three potential PTB-binding sites allowing for an interaction with substrates like IRS-1 (38) and IRS-2. The present data clearly show that EGF increases the tyrosine phosphorylation of the same signaling molecules as seen with insulin and, in addition, Gab1 as well as its cognate receptor. Furthermore, both insulin and EGF increase the downstream phosphorylation and activation of PKB/Akt, which seems to play an important role for the insulin-stimulated glucose transport (8, 9), whereas EGF increased MAP kinase phosphorylation to a greater extent than insulin.

The finding that the EGFR is also abundantly expressed in human skeletal muscle is consistent with our preliminary finding that EGF, similar to the results in fat cells, increases glucose transport in muscle to the same extent as insulin. These findings raise the possibility that EGF, by virtue of its ability to activate a multitude of docking proteins for PI 3-kinase (IRS-1, IRS-2, Gab1, and EGFR), may modify the metabolic response in insulin-resistant states, including Type 2 diabetes, where PI 3-kinase activation and tyrosine phosphorylation of IRS-1 are markedly reduced in both fat cells (26, 32) and skeletal muscle (39). Our findings clearly support this possibility, since EGF was able to augment downstream insulin signaling in cells from Type 2 diabetic subjects.

The binding specificity of the EGF family of ligands for the EGFR family members are classified into different groups (40). One group, which binds to EGFR homodimers, includes EGF and TGF-α, whereas the heregulins bind to Erbβ3 and Erbβ4. The present findings that both EGF and TGF-α elicit the metabolic effects, combined with our finding that AG1478, a specific kinase inhibitor of EGFR, inhibited the effects of EGF (data not shown), clearly show that they were mediated through EGFR. The phosphorylation and activation of Gab1 by EGF in human fat cells is similar to what has been reported in other cells, although Gab2 has also recently been reported to be the major docking protein for PI 3-kinase activation in isolated rat hepatocytes (21). However, both Gab2 and Shc were only slightly and inconsistently phosphorylated by EGF in human fat cells.

Gab1, which belongs to the insulin receptor substrate-like family of adaptor molecules (20), is phosphorylated by several growth factors (including EGF, hepatocyte growth factor, nerve growth factor, and PDGF), cytokines (including IL-3 and IL-6, interferon α and β, erythropoietin, and thrombopoietin), and T and B cell antigens (18, 20, 41, 42). Despite this lack of specificity, Gab1 has been found to play a critical role for hepatocyte growth factor signaling, and Gab1-deficient mice display a phenotype that is similar to that seen in hepatocyte growth factor null mice (43).

Gab1 was a major tyrosine-phosphorylated band in response to EGF in human fat cells from both healthy and Type 2 diabetic subjects. Gab1 activation is associated with the recruitment of several SH2-containing signal transducers like SHPTP-2, PI 3-kinase, phospholipase C-γ, and Shc, factors generally linked to mitogenesis and cell growth. Whether Gab1 activation, in addition to IRS-1 and IRS-2 (4), also can play a role for the insulin-like effects of EGF (glucose transport and antilipolysis) remains to be established. However, the present data suggest that this is possible, since EGF-stimulated Gab1 phosphorylation was normal in diabetic cells and EGF also augmented the downstream insulin signaling (Ser(P)-PKB) in these cells.

The finding that EGF elicits the phosphorylation of both IRS-1 and IRS-2 is in agreement with the recent work of Fujikawa et al. in cultured rat hepatocytes as well as A431 cells abundantly expressing EGF (38, 44). These authors reported that EGF directly phosphorylated IRS-1 in in vitro assays (44) and that this was markedly attenuated if the C-terminal of EGFR was truncated. The human EGFR contains three C-terminal NPXY sites, which could bind to the PTB domains of IRS-1, similar to the insulin receptor (45).

The fact that the insulin-mimicking effects of EGF has not
been reported before and that discrepant data have been reported in 3T3-L1 cells overexpressing EGFR (14, 15) is probably due to the different cell types studied as well as the rapid time of onset of EGFR. Peak phosphorylation of IRS-1 is very rapid (within 2 min) and then declines (44). In human fat cells, where EGFR is highly abundant, we could confirm a rapid onset (<2 min), but the tyrosine phosphorylation of IRS-1 remained for at least 15 min.

The tyrosine phosphorylation of IRS-1 and IRS-2 is probably due to kinase activity of EGFR and the binding to the PTB domain of IRS-1 and IRS-2. Further studies aimed at clarifying the interaction of JAK2 with IRS-1 following EGFR, although JAK2 showed some degree of tyrosine phosphorylation (data not shown).

An intriguing question, then, is why highly differentiated human fat cells maintain a highly abundant expression of EGFR that activate both the metabolic and growth-promoting pathways. Since human skeletal muscle also shows a high level of expression, a relevant question is whether EGF/EGFR can play a role in human pathophysiology or whether they are merely remnants from the growth and development of the skeletal muscle and adipose tissue. However, EGFR has been shown to markedly increase the growth and proliferation of primordial cells in the adipose tissue of rodents (46). Similarly, we have found EGFR to markedly increase the growth of a human preadipocyte cell line. Thus, EGF/EGFR may play an important role in early adipose tissue growth.

We found no evidence that EGF or TGF-α are expressed in human fat cells or skeletal muscle exhibiting a local autocrine function. However, since EGFR have a high affinity for the ligands, circulating levels of EGF (around 60–180 pM) (47) may play a role in glucose uptake and lipid mobilization in humans and may, for instance, explain why human subjects with mutations making the insulin receptors nonfunctional do not develop severe, ketotic diabetes like animal models devoid of insulin receptors (48, 49). Further studies aimed at clarifying the role of EGF in the adipose tissue are currently in progress.

In conclusion, the present data show that growth factors like EGF/EGF-α are capable of mimicking the effects of insulin in human fat cells and that EGF receptors are abundantly expressed in both human skeletal muscle and fat cells. Furthermore, EGF activates several PI 3-kinase pools (IRS-1, IRS-2, Gab1, and EGFR) that are additional to those of insulin (IRS-1/2). This ability makes it possible for EGF to augment the effects of insulin in insulin-resistant states like Type 2 diabetes.

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