Fibroblast Growth Factor 1
A Key Regulator of Human Adipogenesis

Louise Hutley,1,2 Wenda Shurety,1 Felicity Newell,1 Ross McGeary,3 Nicole Pelton,1 Jennifer Grant,1 Adrian Herington,4 Donald Cameron,5 Jon Whitehead,1 and Johannes Prins1,2

Obesity, with its related problems, is recognized as the fastest growing disease epidemic facing the world, yet we still have limited insight into the regulation of adipose tissue mass in humans. We have previously shown that adipose-derived microvascular endothelial cells (MVECs) secrete a factor(s) that increases proliferation of human preadipocytes. We now demonstrate that coculture of human preadipocytes with MVECs significantly increases preadipocyte differentiation, evidenced by dramatically increased triacylglycerol accumulation and glycerol-3-phosphate dehydrogenase activity compared with controls. Subsequent analysis identified fibroblast growth factor (FGF)-1 as an adipogenic factor produced by MVECs. Expression of FGF-1 was demonstrated in MVECs but not in preadipocytes, while preadipocytes were shown to express FGF receptors 1–4. The proliferative effect of MVECs on human preadipocytes was blocked using a neutralizing antibody specific for FGF-1. Pharmacological inhibition of FGF-1 signaling at multiple steps inhibits preadipocyte replication and differentiation, supporting the key adipogenic role of FGF-1. We also show that 3T3-L1 cells, a highly efficient murine model of adipogenesis, express FGF-1 and, unlike human preadipocytes, display no increased differentiation potential in response to exogenous FGF-1. Conversely, FGF-1–treated human preadipocytes proliferate rapidly and differentiate with high efficiency in a manner characteristic of 3T3-L1 cells. We therefore suggest that FGF-1 is a key human adipogenic factor, and these data expand our understanding of human fat tissue growth and have significant potential for development of novel therapeutic strategies in the prevention and management of human obesity. Diabetes 53:3097–3106, 2004

From the 1Department of Diabetes and Endocrinology and University of Queensland Department of Medicine, Princess Alexandra Hospital, Woolloongabba, Australia; the 2Adipogen, University of Queensland, St. Lucia, Australia; the 3Chemistry Department and School of Pharmacy, University of Queensland, Brisbane, Australia; the 4Centre for Molecular Biotechnology, School of Life Sciences, Queensland University of Technology, Brisbane, Australia; and the 5Princess Alexandra Hospital Centre for Health Research, Princess Alexandra Hospital, Woolloongabba, Australia.

Address correspondence and reprint requests to Dr. Louise Hutley and Prof. Johannes Prins, Department of Diabetes and Endocrinology and University of Queensland Department of Medicine, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Qld 4102, Australia. E-mail: lhutley@soms.uq.edu.au and jprins@soms.uq.edu.au.

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J.P. is the Scientific Director of Adipogen.

ECGF, endothelial cell growth factor; FGF, fibroblast growth factor; FGFR, FGF receptor; G3PDH, glycerol-3-phosphate dehydrogenase; MVEC, microvascular endothelial cell; PPAR, peroxisome proliferator–activated receptor; TZD, thiazolidinedione.

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It is established that prevention or treatment of obesity plays a significant role in reducing rates of cardiovascular disease, type 2 diabetes, and orthopedic disease, all of which impact considerably on health care and social costs. To develop more sustainable therapies for the treatment of obesity, a better understanding of human adipose tissue growth is essential.

Expansion of adipose tissue in obesity is preceded by the development of new capillaries (1). These new capillaries are formed by the migration and replication of microvascular endothelial cells (MVECs) from established blood vessels (2). Studies in fetal adipose tissue suggest both spatial and temporal relationships between adipogenesis and angiogenesis (3). Therefore, paracrine interaction between MVECs in the developing capillary network and preadipocytes resident in the adipose tissue depots may play a role in the regulation of adipose tissue growth.

We have previously reported that MVECs derived from human adipose tissue secrete a factor(s) that stimulates proliferation in human subcutaneous and omental (intraabdominal) preadipocytes (4). This proliferative effect of MVECs on human preadipocytes was significantly greater from endothelial cells isolated from adipose tissue than from dermally derived MVECs (4), suggesting that endothelial cells from human adipose tissue have unique activities necessary for preadipocyte proliferation.

For an increase in adipose tissue mass to occur, proliferation of preadipocytes needs to be followed by differentiation of these cells to the mature adipocyte phenotype (5). This is an energy-requiring, regulated process characterized in vitro by the sequential acquisition of protein expression patterns and morphological features (6). Intrinsic to the process is the development of the potential to store energy in the form of triglyceride, development of the capacity for insulin-stimulated glucose uptake, and production of characteristic proteins such as glycerol-3-phosphate dehydrogenase (G3PDH) (7) and leptin (8,9). While the transcription factors peroxisome proliferator–activated receptor (PPAR)-γ and CCAAT enhancer binding protein-α are known to be master regulators of this differentiation process (10), factors affecting the earliest stages of adipocyte differentiation are yet to be defined. Furthermore, elucidation is needed of the factors regulating the mechanisms involved in cellular, metabolic, and regional differences among adipose tissue depots and how these factors may impact on the adipocyte hyperplasia characteristic of massive obesity.
We therefore further explored the effects of adipose-derived MVECs on human adipogenesis using coculture techniques and demonstrated that MVECs not only stimulate proliferation of preadipocytes but also markedly increase their differentiation potential. Subsequent work identified a member of the fibroblast growth factor (FGF) family as a factor having profound effects on human preadipocyte proliferation and differentiation in vitro.

**RESEARCH DESIGN AND METHODS**

Unless otherwise stated, all chemicals used were obtained from Sigma-Aldrich (Castle Hill, Australia). Goat anti-human polyclonal FGF-1 neutralizing antibody was purchased from R&D Systems (Minneapolis, MN). Mouse anti-human monoclonal FGF-1 antibody (Sigma) was used in Western blotting procedures. The thiazolidinedione (TZD) compound rosiglitazone was supplied by GlaxoSmithKline (Middlesex, U.K.). The FGF inhibitory peptide Ac-ValTyrMetSerProPhe-NH₂ was prepared by manual synthesis using Fmoc chemistry on Rink Amide MBHA resin. After washing and drying, the peptide was cleaved using TFA/water/triisopropylsilane (95:2.5:2.5) and purified by reverse-phase high-performance liquid chromatography on a Vydac C-18 column using aqueous acetonitrile containing 0.1% formic acid.

Paired omental (intra-abdominal) and subcutaneous adipose tissue biopsies were obtained from 17 male (average age 63.5 years [range 37–82]); average BMI 27.2 kg/m² [21–39]) and 25 female (average age 51.2 years [range 28–68]; average BMI 30 kg/m² [20.5–47.9]) patients undergoing elective open-abdominal surgery. None of the patients had diabetes or severe systemic illness, and none were taking medications known to affect adipose tissue mass or metabolism. The protocol was approved by the research ethics committees of the Princess Alexandra Hospital and the Queensland University of Technology. All patients gave their written informed consent.

**Isolation of stromal-vascular cells.** Preadipocytes, adipocytes, and MVECs were obtained from adipose tissue biopsies as previously reported (4,11,12). Cell culture. Preadipocytes and MVECs were isolated, cultured, and characterized as previously reported (4). Homogeneous cultures of both cell types were grown for up to 8 weeks and maintained at 37°C with 5% CO₂. Cells were used in experimental work at passages 2 and 3. For control and growth factor experiments, preadipocytes were grown in the same serum-containing (10%) medium as the MVECs, containing 90 μg/mL heparin, 0.014 mol/L HEPES, and 0.15% NaHCO₃ (wt/vol), with or without growth factors (all human): β-endothelial cell growth factor (EGF) (at 10 ng/mL) (Sigma), FGF-1, FGF-2, or IGF-1 (at 1 ng/mL) (R&D Systems). In some experiments, FGF inhibitors (anti-FGF-1 neutralizing antibody; R&D Systems) (10 μg/mL), the synthetic peptide Ac-ValTyrMetSerProPhe-NH₂ (10 μg/mL), and suramin (20 μmol/L) were used in the presence of FGF-1 to determine the effects on both proliferation and differentiation. Human dermal fibroblasts were isolated and cultured as previously described (13). These cells were used as controls in the coculture studies.

**MVECs plus preadipocytes.** For coculture experiments, preadipocytes were seeded at low cell density with homogeneous MVECs (each cell type plated at approximately one-third confluence at opposite ends of the culture dish) and the mixed cultures allowed to grow together for 2–6 weeks in endothelial cell growth medium containing heparin and 10 ng/mL β-ECGF. Controls included preadipocytes plus human skin fibroblast cocultures and preadipocyte-only cultures grown under the same conditions as the MVEC plus preadipocyte cultures.

**3T3-L1 adipocytes.** 3T3-L1 fibroblasts were grown to confluence in serum-containing medium consisting of Dulbecco’s modified Eagle’s medium/HAM’s F12 (1:1) (ICN Biomedical Australasia) containing 4.5 g/L glucose, 10% FCS, 100 IU penicillin, 100 μg/mL streptomycin, and 2 mmol/L glutamine. In some experiments FGF-1, FGF-2, or IGF-1 (all at 1 ng/mL) (R&D Systems) were included in the growth medium. Differentiation was initiated by addition of 250 mmol/L dexamethasone, 0.5 mmol/L of 1-methyl-3-isoubutylxanthine, 1 μmol/L insulin, and 0.1 μmol/L rosiglitazone. After 2 days, cells were transferred to serum-containing medium containing 0.5 μmol/L insulin and refed twice weekly. Cells were studied at 13–19 days postdifferentiation. To determine the role of endogenous FGF-1 in 3T3-L1 differentiation, cells were treated with anti–FGF-1 neutralizing antibody (10 μg/mL) for 1 week before and throughout the differentiation period and effects assessed by G3PDH activity assay.

**Adipose differentiation protocol.** As previously reported, confluent preadipocytes at passage 2 were changed to a chemically defined serum-free medium with or without rosiglitazone for induction of differentiation (4,6,12–14). In some experiments, growth factors were included in the differentiation medium at a concentration of 1 ng/mL.

**Assessment of adipose differentiation.** After 21 days in serum-free differentiation medium, adipogenesis was assessed using a Nile Red assay for triacylglycerol accumulation and an enzyme assay measuring G3PDH activity (4,12,13). The Nile Red results are presented as optical density units at 480/540 nm.
Preadipocyte proliferation assay. Following various treatments, proliferation of preadipocytes was assessed using an MTS proliferation assay (Promega, Madison, WI) as described previously (4).

Electrophoresis and Western blot analysis. FGF-1 expression was determined in whole-cell protein isolated from human preadipocytes treated or not treated with FGF-1, human adipose–derived MVECs, 3T3-L1 preadipocytes (all these cell types were maintained in 10% serum-containing medium before protein extraction), and isolated human adipocytes. Cells were harvested in buffer containing 20 mmol/l HEPES, 1 mmol/l EDTA, phosphatase inhibitors (2 mmol/l Na3VO4, 1 mmol/l Na4P2O7, and 10 mmol/l NaF), and protease inhibitors (Complete Mini; Roche Diagnostics, Mannheim, Germany). Whole-cell lysates (15 μg) were resolved on SDS-polyacrylamide gels and transferred to Immobilon-P PVDF membranes (Millipore, Bedford, MA). Membranes were blocked with 2% (wt/vol) BSA/PBST overnight at 4°C, incubated with mouse anti-human monoclonal FGF-1 antibody (Sigma), and probed with horseradish peroxidase–linked secondary antibodies (Amersham) for 1 h at room temperature. Proteins were detected by enhanced chemiluminescence substrate (Amersham).

RT-PCR. Total RNA was obtained from both human subcutaneous adipose tissue and preadipocytes (at confluence and following 21 days of differentiation). Total RNA was obtained using the TRIzol reagent extraction procedure (GIBCO/BRL) according to the manufacturer’s instructions. RNA was further purified using an RNAeasy column (Qiagen) as described by the manufacturer. An on-column DNase digest was performed as part of the purification protocol. Total RNA (1 mg) was reverse transcribed using random hexamers and reverse transcription reagents from Applied Biosystems (Victoria). The

The homogenate was passed through a 21-gauge needle 10 times and a 22-gauge needle 4 times. The samples were cleared by centrifugation at 1,000g for 10 min at 4°C. Protein determination was performed using bicinchoninic acid reagent (Pierce, Rockville, IL). Proteins (15 μg) were resolved on SDS-polyacrylamide gels and transferred to Immobilon-P PVDF membranes (Millipore, Bedford, MA). Membranes were blocked with 2% (wt/vol) BSA/PBST overnight at 4°C, incubated with mouse anti-human monoclonal FGF-1 antibody (Sigma), and probed with horseradish peroxidase–linked secondary antibodies (Amersham) for 1 h at room temperature. Proteins were detected by enhanced chemiluminescence substrate (Amersham).

FIG. 2. Effect of growth factors on human preadipocyte differentiation, as measured by G3PDH activity. A: Six weeks with or without β-ECGF and in the absence or presence of coculture with MVECs before differentiation (subcutaneous, n = 6; omental, n = 5; *P > 0.1 for both subcutaneous and omental) (medium 1, serum-containing preadipocyte growth medium; medium 2, medium 1 plus 10 ng/ml β-ECGF). B: FGF-1 and -2 are potent adipogenic agents (FGF-1 more than FGF-2 in subcutaneous preadipocytes, P = 0.003, n = 3), whereas no significant effect was demonstrated for IGF-1. C: The adipogenic effects of FGF-1 occurred subsequent to exposure of preadipocytes during proliferation. This effect was further enhanced when treatment with FGF-1 was maintained throughout differentiation (n = 3, P = 0.004 vs. proliferation only).
absence of contaminating genomic DNA was verified by including samples to which no reverse transcriptase was added (no reverse transcriptase controls). For PCR, no template controls confirmed the absence of PCR contamination. Fibroblast growth factor receptor (FGFR)-1 to -4 primer sequences were those used by Tartaglia, Fragale, and Battaglia (15). Thermal cycling conditions were as follows: first denaturing step of 94°C for 5 min; 35 cycles of 94°C for 45 s, 60°C for 30 s, and 72°C for 30 s; and a final extension step of 72°C for 10 min. FGFR cDNA (in pcDNA3.1) was a kind gift from Pamela Maher (Scripps Research Institute, La Jolla, CA) and was used as a positive control for each reaction.

**Statistics.** Data were analyzed using repeated-measures ANOVA for differences across experimental groups. Student’s t test was used to evaluate the significance of the difference in mean values between different treatments. Data are expressed as means ± SE.

**RESULTS**

**Adipocyte differentiation.** Human MVECs and preadipocytes were isolated, cultured, and characterized as previously described (4). Once phenotype was established, these two cell types were used in coculture experiments to study involvement of MVECs in human adipogenesis.

At confluence following a 2-week coculture period, all cultures were changed to serum-free medium containing known inducers of adipocyte differentiation, including the PPARγ ligand rosiglitazone. In preadipocyte-only cultures, in accordance with our previous observations using standard human differentiation techniques (13), lipid accumulation was apparent in a small number of cells by day 7–10, with 5–25% of cells acquiring lipid by 21 days (Fig. L A). In marked contrast, triacylglycerol accumulation was apparent as early as day 3–5 in the majority of cocultured preadipocytes, with 70–95% of cells accumulating large amounts of triacylglycerol by day 21 (Fig. 1 B). Evidence for the specificity of these effects for human preadipocytes came from observations showing that human skin fibroblasts cultured either alone or in coculture with MVECs, under identical conditions as the preadipocytes, did not accumulate lipid when treated with differentiation medium (medium 1) or the same medium with added β-ECGF (medium 2; β-ECGF is required for maintenance of endothelial cells in the coculture conditions). This was the case for preadipocytes from both subcutaneous (G3PDH, Fig. 1 C; triacylglycerol, Fig. 1 D) and omental (G3PDH, Fig. 1 E; triacylglycerol, Fig. 1 F) adipose tissue depots. While the rise in G3PDH activity in subcutaneous preadipocytes was greater than that seen in cells from omental tissue, the degree of differentiation of omental cells in coculture was significant. To our knowledge, this represents the first time quantifiable and consistent differentiation that has been demonstrated in intraabdominal preadipocytes following prolonged culture in the presence of serum. After treatment with the same differentiation medium as human preadipocytes, G3PDH activity was negligible in human skin fibroblasts cocultured with adipose-derived MVECs (data not shown).

**FGF-1 in human adipogenesis.** To determine whether the differentiation potential of human preadipocytes could be further enhanced, coculture with MVECs was extended to a period of 6 weeks before induction of differentiation. Following 21 days in differentiation medium, after the coculture period, both G3PDH activity and triacylglycerol accumulation were once again significantly increased in cocultured preadipocytes over that demonstrated in preadipocytes previously maintained as homogeneous cultures in regular serum-containing growth medium (medium 1) (G3PDH, Fig. 2 A). Surprisingly, however, both parameters of differentiation were also elevated to the same high levels as those measured in the cocultured cells in preadipocyte-only cultures maintained for 6 weeks in serum-containing medium supplemented with β-ECGF (medium 2) (Fig. 2 A). These results suggest that β-ECGF is acting as an adipogenic agent under these conditions. The presence of heparin in medium 2 was required for maximal β-ECGF activity, but heparin, in the absence of growth factor, had no effect on differentiation of human preadipocytes (data not shown).

β-ECGF is a member of the FGF family and is a precursor molecule of FGF-1 (16). To further investigate the role of growth factors in this system, differentiation of preadipocytes was carried out following a 6-week proliferation period with or without FGF-1, FGF-2, or IGF-1 (at 1 ng/ml). Results demonstrated that pretreatment with FGF-1 and FGF-2 but not IGF-1 resulted in significant
increases in G3PDH activity in human preadipocytes, relative to controls, with FGF-1 having the greatest effect in subcutaneous preadipocytes (Fig. 2H). The differentiation potential of these cells could be further enhanced if exposed to FGF-1 during both proliferation and differentiation (Fig. 2C). Intriguingly, preadipocytes treated with FGF-1 only during the differentiation period exhibited a trend to a decrease in both G3PDH activity (Fig. 2C) and lipid accumulation following differentiation compared with cells grown in the absence of FGF-1. The time course of action of FGF-1 suggests that its effect is to prime preadipocytes for subsequent differentiation rather than to act as a direct inducer of differentiation.

**Preadipocyte proliferation.** The proliferation rate of human preadipocytes is increased in response to FGF-1, and this effect can be blocked by addition of a commercially available neutralizing antibody raised against FGF-1 (Fig. 3). To establish more directly whether FGF-1 may represent an adipogenic factor produced by MVECs, we used the same neutralizing anti–FGF-1 antibody to block any endogenous FGF-1 actions in MVEC-conditioned medium. Proliferation assays were carried out and, in agreement with our previous results (4), preadipocyte proliferation was enhanced in response to medium that had been conditioned by 48-h exposure to adipose-derived MVECs from both subcutaneous and omental sites (Fig. 3). Inclusion of the FGF-1 neutralizing antibody at 10 μg/ml to the MVEC-conditioned medium resulted in almost total abrogation of these proliferative effects (Fig. 3).

**PPAR-γ involvement in FGF-1–induced adipogenesis.** Significant, but relatively small, increases in G3PDH activity were demonstrated in FGF-1–treated preadipocytes from both subcutaneous and omental adipose tissue differentiated in the absence of PPAR-γ ligand (Fig. 4). However, the maximal adipogenic effect of either coculture with MVECs or treatment with FGF-1 was only observed when cells were differentiated in the presence of the PPAR-γ ligand rosiglitazone (a TZD) (Fig. 4). Differentiation in the presence of the TZD exhibited depot specificity, in agreement with our previous results and those of others (13,14,17), with the effect being greater in subcutaneous than omental preadipocytes (Fig. 4). The requirement for TZD for maximal differentiation suggests that neither the factor(s) produced by MVECs nor exogenous FGF-1 are acting as PPAR-γ ligands. Further evidence for this came from electromobility shift and reporter construct assays, which showed no endogenous PPAR response element binding or activation of PPAR-γ (data not shown) in response to FGF-1 treatment, and TZD-induced PPAR-γ activity was not further enhanced in the presence of FGF-1 (data not shown).

**FGF-1 expression in human adipose tissue.** Using a combination of Western blot analysis and immunofluorescence, robust expression of FGF-1 was demonstrated in adipose-derived MVECs and 3T3-L1 cells. In contrast, expression in human preadipocytes, maintained under the same conditions, was low to undetectable (although this does not rule out the possibility of expression at very low levels). Expression in isolated human adipocytes was more variable than in preadipocytes but, if detectable, was always lower than that seen in either 3T3-L1 cells or human MVECs (Fig. 5). Gabrielson et al. (18) demonstrated FGF-1 expression in the stromovascular fraction of human adipose tissue, which contains both MVECs and preadipocytes. Our findings suggest that the FGF-1 expression in this adipose tissue fraction is predominantly contributed to by the MVEC component.

**FGFR expression in human adipose tissue.** FGFR expression in human samples was determined using RTPCR (Fig. 6). In subcutaneous adipose tissue, expression of FGFR-1 and -2 was detected. Similar expression patterns were demonstrated in differentiated human preadipocytes, with R1 and R2 being present, whereas little, if any, expression of R3 and R4 was detected. In contrast, all four FGFRs were found to be expressed in nondifferentiated human preadipocytes (Fig. 6). Western blotting and immunofluorescence techniques also demonstrated expression of FGFR-1 to -4 in human nondifferentiated preadipocytes (data not shown). In 3T3-L1 cells, expression of all four receptors has been observed by RT-PCR or Western blotting (data not shown).

**Effects of FGF inhibitors on human preadipocyte proliferation and differentiation.** To determine whether the adipogenic effects of FGF-1 can be blocked in vitro, preadipocytes were treated with a combination of FGF-1 and FGF inhibitors and effects on both proliferation and differentiation assessed. The inhibitors used included the synthetic peptide Ac-ValTyrMetSerProPhe-NH₂ (peptide 1), which specifically binds to, and blocks, FGFRs (19); suramin (a polysulfonated binaphthyl urea that inhibits complex formation among FGF, heparin, and cognate receptor) (20); and a neutralizing antibody specific for FGF-1 (data not shown).
FGF-1 (21). All of these compounds are reported to inhibit the mitogenic activity of FGF-1 (19–21). All three compounds demonstrated significant inhibition of FGF-1 proliferative effects (Fig. 3, anti–FGF-1; Fig. 7A, peptide 1 and 7B, suramin), while suramin and anti–FGF-1 neutralizing antibody also demonstrated inhibition of differentiation (Fig. 7C, suramin and 7D, anti–FGF-1). These data support the role of FGF-1 as an adipogenic agent and show that the adipogenic effects can be blocked in vitro.

Effects of growth factors on 3T3-L1 proliferation and differentiation. Investigation of adipogenic effects of growth factors was also carried out using the 3T3-L1 murine model of adipogenesis which, unlike human preadipocytes, display rapid growth and high differentiation capacity. Exposure of 3T3-L1 fibroblasts to FGF-1, FGF-2, or IGF-1, before differentiation, caused no significant increases in either growth, G3PDH activity, or triacylglycerol accumulation (data not shown) compared with the already high levels of both parameters seen in the absence of growth factors (controls). For the FGF-1 and -2 treatments, this was in direct contrast to results in human preadipocytes. Western blot analysis demonstrated expression of FGF-1 in 3T3-L1 fibroblasts but not in human preadipocytes (Fig. 5). Also, treatment of 3T3-L1 cells with anti–FGF-1 neutralizing antibody for 1 week before and throughout differentiation resulted in a significant decrease in G3PDH activity following differentiation compared with controls (Fig. 7E). Hence the expression of FGF-1 by the embryonically derived 3T3-L1 cells may underpin their capacity to differentiate more readily in vitro than human preadipocytes.

Differentiation of human preadipocytes in the presence of serum. In contrast to 3T3-L1 cells, human preadipocytes are slow growing and differentiate inefficiently in vitro. Human preadipocytes also have more defined and specific requirements for induction of differentiation than 3T3-L1 cells, including an absolute requirement for serum-free conditions (6). Following treatment with FGF-1, however, we observe that human preadipocytes display rapid growth characteristics and a high capacity for differentiation that are more similar to 3T3-L1 cells than to non–FGF-1–treated preadipocytes. To determine whether growth factor treatment of human preadipocytes renders them capable of differentiating in the presence of serum, cells were proliferated in the presence of FGF-1, FGF-2, or IGF-1 and induced to differentiate in serum-containing medium. Using a 3T3-L1 differentiation protocol consisting of serum-containing medium (10% vol/vol), insulin, and, for the first 3 days, glucocorticoid and IBMX, FGF-1–treated preadipocytes from both subcutaneous and omental sites developed both morphological (Fig. 8A) and biochemical (Fig. 8B) features of a differentiated phenotype. The effect of FGF-2 treatment during growth also lead to an increased capacity for differentiation in the presence of serum, but this effect, although similar to FGF-1 treatment for omental preadipocytes, was much less in subcutaneous preadipocytes than that measured in FGF-1–treated cells. Preadipocytes treated with IGF-1 were similar in differentiation capacity to controls, with no lipid accumulation and negligible G3PDH activity (Fig. 8B). In our experience, this is the first time preadipocyte differentiation has been observed in cells maintained for an extensive period in, and subsequently differentiated in, the presence of serum.

DISCUSSION

Knowledge of the cellular mechanisms implicated in the overdevelopment of adipose tissue is a critical issue in the design of pharmaceutical strategies aimed at lessening nutritional obesity. We have previously reported that adipose-derived MVECs produce factors that have a mitogenic effect on human preadipocytes. We now demonstrate that these endothelial cells also secrete factors that significantly increase the differentiation potential of preadipocytes from both subcutaneous and omental adipose tissue depots (subcutaneous more than omental). We present data that indicate that much of the adipogenic effect of MVECs can be attributed to FGF-1 secretion.

FGF-1 is a member of a large family (currently >22

FIG. 6. FGFR expression in adipose cells. RT-PCR analysis demonstrates expression of mRNAs for FGFR-1 to -4 in human nondifferentiated preadipocytes. No mRNA expression of FGFR-3 and -4 was detected in human whole adipose tissue (subcut) or differentiated preadipocytes. Lane 1: No template control; lane 2: pcDNA3.1 (FGFR-1 to -4, positive control); lane 3: human adipose tissue (subcut); lane 4: human nondifferentiated preadipocytes; lane 5: human differentiated preadipocytes/adipocytes.
differentiated in the presence of a PPAR-
made much more apparent if the cells are subsequently
prime the cells for differentiation. This priming effect is
FGF-1 from the time of preadipocyte isolation appears to
human adipogenic molecule. Culture in the presence of
transduction molecules and pathways (28,29).
and/or phosphorylation of multiple downstream signal
in response to FGFs occurs through receptor dimerization
the intracellular domain of the FGFR initiates recruitment
cans (26). Subsequent phosphorylation at multiple sites on
specificity by heparan sulfate proteoglycans (23,25–27). In addition to localization
FGFs and FGFRs, and modulation of binding affinity and
receptors, modulation of binding specificity by alternative
splicing of FGFRs and by sequence differences between
receptors, modulation of binding specificity by alternative
FGFs, including tissue-specific expression of ligands and
receptors, modulation of binding affinity by alternative
splicing of FGFRs and by sequence differences between
FGFs and FGFRs, and modulation of binding affinity and
specificity by heparan sulfate molecules with specific
patterns of sulfation (23,25–27). In addition to localization
in the plasma membrane, FGFRs are also expressed within
the nuclear envelope and matrix (28). Signal transduction
in response to FGFs occurs through receptor dimerization
and complex formation with heparan sulfate proteoglycans (26). Subsequent phosphorylation at multiple sites on
the intracellular domain of the FGFR initiates recruitment
and/or phosphorylation of multiple downstream signal
transduction molecules and pathways (28,29).
This study demonstrates a novel role of FGF-1 as a key
human adipogenic molecule. Culture in the presence of
FGF-1 from the time of preadipocyte isolation appears to
prime the cells for differentiation. This priming effect is
made much more apparent if the cells are subsequently
differentiated in the presence of a PPAR-γ ligand, the TZD
rosiglitazone. Our results suggest that FGF-1 is not acting
as, or promoting production of, a PPAR-γ ligand. It is
possible, however, that FGF-1 treatment of preadipocytes
may be acting on the PPAR-γ system in other ways. These
could include increased expression of PPAR-γ, as well as
members of the CCAAT enhancer binding protein family of
transcription factors, before induction of differentiation.
This would be consistent with the finding that FGF-1
primes preadipocytes for TZD-induced differentiation.
The adipogenic effects of MVECs and FGF-1 were
specific for cells obtained from the stromal-vascular
compartment of adipose tissue because human skin fibroblasts
maintained under identical conditions did not accumulate
cytoplasmic lipid and no G3PDH activity was measured in
preadipocytes in response to FGF-1 is also abro-
gated in the presence of FGF inhibitors. G3PDH
activity was assessed in differentiated preadipo-
cyes following exposure, both before and during
differentiation, to FGF-1 and the inhibitors suramin
(20 μmol/l) (n = 6, *P < 0.001) (C) and specific
neutralizing anti–FGF-1 antibody (anti-FGF-1) (10
μg/ml) (n = 6, *P < 0.001) (D) and specific
neutralizing anti–FGF-1 antibody from 10 days
before induction through day 12 of differentiation
results in a decrease in G3PDH activity in differen-
tiated cells versus controls (n = 3, *P = 0.05).
(Inserts in A and B show results for the FGF-1 and
FGF-1 plus inhibitor treatments [A, peptide 1; B, suramin], presented as the increase/decrease in ab-
sorbance units versus non–FGF-1–treated cells.)

**FIG. 7.** FGF-1 inhibition results in decreases in
preadipocyte proliferation and/or differentiation.
MTS assay demonstrates increases in proliferation
rate of FGF-1–treated human preadipocytes versus
non–FGF-1–treated controls (Ctrl) (A and B: *P < 0.05 for control vs. FGF-1). This FGF-1–induced
growth is attenuated in the presence of the FGF
inhibitors peptide 1 (Pep 1) (10 μg/ml) (n = 6, *P = 0.03) (A) and suramin (Sur) (20 μmol/l) (n = 6,
*P = 0.02) (B). Data in A and B are presented as
both raw data (490 nm) (which shows control value)
and (see inserts) as increase/decrease in absorb-
bance units versus control. Differentiation of human
preadipocytes in response to FGF-1 is also abro-
gated in the presence of FGF inhibitors. G3PDH
activity was assessed in differentiated preadipo-
cyes following exposure, both before and during
differentiation, to FGF-1 and the inhibitors suramin
(20 μmol/l) (n = 6, *P < 0.001) (C) and specific
neutralizing anti–FGF-1 antibody (anti-FGF-1) (10
μg/ml) (n = 6, *P < 0.001) (D). E: Exposure of
non–FGF-1–treated 3T3-L1 preadipocytes to a spe-
cific anti–FGF-1 neutralizing antibody from 10 days
before induction through day 12 of differentiation
results in a decrease in G3PDH activity in differen-
tiated cells versus controls (n = 3, *P = 0.05).
(Inserts in A and B show results for the FGF-1 and
FGF-1 plus inhibitor treatments [A, peptide 1; B, suramin], presented as the increase/decrease in ab-
sorbance units versus non–FGF-1–treated cells.)
of TZD. In contrast, and consistent with our previous reports and those of others (13,14,30,31), differentiation was greater in subcutaneous compared with omental preadipocytes when a TZD was included. Taken together, these observations suggest that subcutaneous and omental preadipocytes may share similar capacity to differentiate in the absence of TZDs and that subcutaneous preadipocytes are more responsive to the adipogenic effects of TZDs than their omental counterparts. This difference may contribute to the observed remodeling of adipose mass, with increased subcutaneous and decreased omental fat, in humans receiving TZDs (32,33). It is also noteworthy that before this work, omental preadipocytes that have been cultured in the presence of serum have proved refractory to differentiation in vitro (13,14). We have now shown that exposure of these cells to FGF-1 during proliferation results in marked differentiation, thus overcoming the serum-induced inhibition of differentiation. Indeed, in FGF-1–treated omental preadipocytes, the levels of G3PDH activity and intracellular lipid accumulation are comparable to those we have previously reported in subcutaneous preadipocytes (13).

The murine 3T3-L1 cell line is a widely used model system for the study of differentiation. In contrast to human preadipocytes, these cells differentiate with high efficiency in the presence of serum and after high passage number (typically up to 20 in our experience). We report that 3T3-L1 cells, unlike human preadipocytes, express high levels of FGF-1 and that exogenous FGF-1 does not further increase proliferation or differentiation of these cells. We also demonstrate that human preadipocytes treated with FGF-1 resemble 3T3-L1 cells in terms of rapid growth and very high capacity for differentiation. Taken together, these results suggest that an important difference between 3T3-L1 and human preadipocytes is higher endogenous expression of FGF-1 by the 3T3-L1 cells. The key role of FGF-1 in promotion of adipogenesis is demonstrated by the inhibitory action of neutralizing anti–FGF-1 antibodies on both human and 3T3-L1 differentiation.

The role of FGFs in adipogenesis is controversial. Although both positive and negative effects have been reported for different family members, including FGF-1 and -2 (34), in general these growth factors are reported to have a mitogenic effect on preadipocytes while having no effect or even potently inhibiting their differentiation (35–42). In contrast to previous studies, where exposure to FGF-1 was restricted to relatively short periods during either growth or differentiation, we have cultured cells in the presence of FGF-1 for sustained periods (1–2 months). In addition, previous studies have not addressed the effects of sustained culturing in the presence of FGF-1 during the growth period, before the induction of differentiation. We suggest that these differences in protocol underlie the differences in experimental observations. Consistent with this, when preadipocytes were exposed to FGF-1 only during differentiation, as in earlier studies, we observed no significant effect on either lipid accumulation or G3PDH activity, with a trend toward a decrease in these markers of differentiation. Taken together, our results suggest that sustained incubation with FGF-1 during the growth period may “prime” the preadipocytes for subsequent differentiation. FGF-2 treatment of human preadipocytes also resulted in increased adipocyte differentiation, suggesting that this feature may be common to members of the FGF family, although FGF-1 was consistently the more potent adipogenic agent, particularly in cells from subcutaneous depots. Further work is aimed at investigation of the role of a range of growth factors in human adipogenesis, including that of FGF-2 and other members of the FGF family.

Evidence that members of the FGF family may play an in vivo role in adipogenesis is provided by studies demon-
strating de novo adipogenesis in mice at the site of implanted FGF-2-impregnated microspheres (43,44). Also, a role for FGFs in human adipose tissue metabolism is suggested by the observation that preadipocytes from massively obese individuals have much higher expression of FGF-2 than the same cells from lean counterparts (45). The fact that human adipose tissue expresses members of the FGF family, including their cognate receptors, provides further support for a metabolic role of these growth factors in human adipose tissue (18,40).

In summary, in this report we describe an important role of adipose-derived MVECs in the paracrine regulation of human adipose tissue expansion and demonstrate a novel role of FGF-1 in human adipogenesis. Our data indicating that disruption of FGF-1 signaling at multiple biochemical steps inhibits preadipocyte replication and/or differentiation support the key adipogenic role of FGF-1 and represent an important area for further research. Future studies will be aimed at establishing the molecular mechanisms by which FGF-1 exerts its adipogenic effects. These data suggest that antagonists of FGF-1 or of FGFs may present new avenues for the development of novel pharmacological antiobesity strategies targeting adipose cell number.

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