Biological Effects of Human Growth Hormone in Rat Adipocyte Precursor Cells and Newly Differentiated Adipocytes in Primary Culture

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The effects of human growth hormone (hGH) on proliferation and differentiation of primary adipocyte precursor cells isolated from rat epididymal fat pads were studied under serum-free culture conditions. hGH markedly reduced the formation of new fat cells and the expression of glycerophosphate dehydrogenase activity, a marker enzyme of adipose differentiation, in a dose-dependent manner. To find an explanation for this inhibitory effect, we investigated the action of GH on (1) cell proliferation and on (2) lipid accumulation, the latter in the absence and presence of corticosterone. In undifferentiated cells, 5 nmol/L hGH increased both cell number and [^{3}H]-thymidine incorporation (1.3- and 2.6-fold over basal, respectively). This effect was mediated by insulin-like growth factor-I (IGF-I), since hGH stimulated IGF-I production in undifferentiated cells by 12-fold and addition of an anti–IGF-I monoclonal antibody (IGF-I MAb) abolished the mitogenic effect of hGH but did not prevent hGH-induced suppression of adipose differentiation. In developing fat cells, hGH significantly reduced cellular 2-deoxyglucose uptake and glucose incorporation into lipids. In addition, hGH exhibited a lipolytic action in the presence of insulin and triiodothyronine. These effects were not prevented by IGF-I MAb. Specific binding of [^{125}I]-hGH to precursor cells increased significantly during adipose conversion. In differentiated cells Scatchard analysis yielded linear plots with an apparent K_d of 0.16 nmol/L and 8,400 sites per cell. Taken together, these data show that hGH reduces adipose conversion in primary cultures of rat adipocyte precursor cells while promoting cell proliferation through an increase in IGF-I production,

GROWTH HORMONE (GH) is an important factor for organ growth and cell differentiation.[1] Moreover, GH exerts a variety of metabolic effects, especially on glucose and lipid metabolism.[2] The clinical observation of a decreased number of fat cells in GH-deficient children suggests that GH might be physiologically important for fat cell development.[3] It is now well established that adipose tissue represents an important target tissue where various actions of GH can readily be seen.[4]

In vitro studies have shown that GH has a stimulating effect on the adipose conversion of several clonal preadipocyte cell lines, such as 3T3- F442A,[5,6] 3T3-L1,[7] and ob1771 and ob17UT.[8] These initially fibroblast-like cells acquire a round shape and accumulate lipids during adipose differentiation. This process is accompanied by the synthesis of a variety of fat cell–specific enzymes until the cells resemble both morphologically and metabolically mature fat cells.[9,10]

Besides this differentiation-promoting action, various metabolic effects of GH have been observed in adipose tissue samples,[11,12] isolated mature fat cells,[13,14] and differentiated 3T3-F442A cells.[15-18] GH exerts two different types of action in adipose tissue. When administered long-term, GH inhibits both lipogenesis and glucose transport and increases lipolysis. In contrast, short-term incubation with GH has been shown to induce transient insulin-like effects.[4,11-18]

Specific binding sites for GH have been demonstrated in previous studies in rat adipocytes,[14,19] ob17 cells,[20] and 3T3-F442A cells.[21] These reports also showed that binding affinity and receptor number are relatively stable during differentiation.

To date, little attention has been paid to the biological effects of GH in mammalian adipocyte precursor cells in primary culture. There are only preliminary reports on the effect of GH on differentiation of primary adipose precursor cells from rat[22] and human tissue,[23] suggesting a lack of GH action in these cells, in contrast to the abovementioned effects in clonal preadipocyte cell lines. However, GH effects may have remained undetected, because these studies were performed under serum-containing culture conditions. Recently, Deslex et al[24] developed a chemically defined serum-free culture system, which renders it possible to investigate biological effects of defined hormones or growth factors in primary cultures of adipocyte precursor cells. Using this culture system, we reexamined the biological effects of human GH (hGH) on adipose differentiation and metabolism in rat adipose tissue.

Since glucocorticoids are known to be adipogenic agents[10,25] and to have opposing effects to GH on differentiation,[26] whereas they augment the lipolytic action of GH,[4] most of the experiments were also performed in the presence of corticosterone.

MATERIALS AND METHODS

Materials

Culture media (Dulbecco's modified essential medium [DMEM] and Ham's F12), fetal calf serum (FCS), newborn calf serum (NCS), Hanks balanced salt solution (HBSS), penicillin-streptomycin, and trypsin-EDTA were obtained from Gibco (Gaithersburg, MD). Tissue culture plasticware was from both Flow Laboratories (Irvine, Scotland) and Gibco (Berlin, Germany). Type I collagenase was purchased from Worthington (Freehold, NJ), and human transferrin, biotin, pantothenate, and bovine serum albumin (BSA) were from Sigma (St Louis, MO). 3-[^{3}H]-D-glucose (specific activ-

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ity, 15 Ci/mmol), [^{3}H]-2-deoxy-D-glucose (specific activity, 14.4 Ci/mmol), and [^{3}H]-thymidine (specific activity, 110 Ci/mmol) were purchased from Amersham (Buckinghamshire, England). A mouse monoclonal antibody (MAb) raised against human insulin-like growth factor-I (IGF-I) was obtained from Serotec (Kidlington, England). All other reagents were of chemical grade and purchased from Sigma. Recombinant hGH, insulin, and IGF-I were kindly provided by Novo Nordisk (Gentofte, Denmark). hGH was labeled with [^{125}I]-iodine to a specific activity of 60 to 80 μCi/μg and purified as previously reported .[27,28]

Cell Preparation and Cell Culture

Stromal-vascular cells from rat adipose tissue were isolated and cultured as described previously,[24] with minor modifications. Epididymal and perirenal fat pads were removed from ad libitum–fed male Wistar rats (body weight, 100 to 140 g). After removal of visible blood vessels, the tissue was finely minced with scissors and digested for 60 minutes at 37°C with collagenase (0.15% wt/vol) in DMEM containing 2% BSA under vigorous shaking. The tissue remnants after digestion represented less than 1% of the starting material. After successive filtration through a 100-μm and 25-μm nylon screen and centrifugation at 400× *g* for 10 minutes, the cell pellet was resuspended in an erythrocyte-lysing buffer consisting of 154 mmol/L NH_4Cl, 10 mmol/L $KHCO_3$, and 0.1 mmol/L EDTA for 10 minutes at room temperature. This treatment led to an almost complete lysis of red blood cells without interfering with cell attachment, growth, or differentiation of nucleus-containing cells in the stromal-vascular fraction, as shown previously.[25] After additional washing and centrifugation steps, the cell pellet was resuspended in DMEM supplemented with 10% FCS and containing 100 U/mL penicillin and 0.1 mg/mL streptomycin. The cells were inoculated into 12-well plates (4.5 cm^2/well) at a density of 5×10^4 cells/cm^2 and maintained in the serum-enriched medium for 12 to 18 hours in a humified atmosphere of 95% air and 5% CO_2 at 37°C. After attachment, cells were then thoroughly washed three times with prewarmed DMEM and finally cultured in a serum-free medium consisting of DMEM/Ham's F12 medium (1:1 vol/vol) supplemented with 15 mmol/L $NaHCO_3$, 15 mmol/L HEPES, 33 μmol/L biotin, 17 μmol/L pantothenate, 100 U/mL penicillin and 0.1 μg/mL streptomycin, and 10 μg/mL transferrin (basal medium). For comparison, some cultures were maintained for the whole culture period in DMEM enriched with 10% FCS or NCS. Culture media were changed every 3 days. hGH was added at concentrations and for periods indicated in the text and figure legends.

Unless stated otherwise, results were obtained with cultures of adipocyte precursor cells from epididymal fat pads. In some experiments, adipocyte precursor cells from perirenal fat pads were also used for comparison and handled as described for the epididymal depots.

Induction of Adipose Differentiation

To induce adipose differentiation, cells were cultured in basal medium supplemented with 1 μmol/L insulin and 200 pmol/L triiodothyronine (IT medium). In other experiments, corticosterone (10 nmol/L) was also present (ITC medium).

Cell Proliferation Experiments

To measure cell proliferation, cells were detached with HBSS containing 0.05% trypsin and 0.02% EDTA and then counted in a hemocytometer. Alternatively, cell number was determined on the culture dishes using a net micrometer (Zeiss, Oberkochen, Germany) at a 100-fold magnification. In the latter case, five different areas each representing 10 mm^2 were counted and the mean cell number per dish was calculated. The two methods yielded comparable results (data not shown).

In addition to the direct cell counts, [^{3}H]-thymidine incorporation was used as a measure of DNA synthesis. For this purpose, rat adipose precursor cells were inoculated in 24-well plates (2 cm^2/well) at a density of 3×10^3 cells/cm^2 in a medium containing 10% FCS. After 12 hours, cells were washed twice with serum-free medium and thereafter maintained in basal medium. Then hGH or IGF-I was added at the concentrations indicated. In some experiments, a mouse antihuman IGF-I MAb was present at a 1:200 dilution. After another 12 hours, [^{3}H]-thymidine (0.2 μCi/well) was added, and the incubation was continued for 12 hours. Then the medium was aspirated, and after being washed twice with cold HBSS, cells were detached with trypsin-EDTA. Finally, cells were harvested using a cell harvester (Wallac, Turku, Finland) and collected on a filter mat. After extensive washing and drying, the filters were counted in a β-scintillation counter.

Morphology

Differentiation of adipocyte precursor cells into mature adipocytes was assessed by conventional microscopy at a 100-fold magnification. Cells were considered differentiated when their cytoplasm was completely filled with lipid droplets. Adipose conversion was additionally assessed by Oil Red O staining. In some experiments, the proportion of differentiated cells in the monolayers was estimated by direct counting using a net micrometer (Zeiss).

Determination of Triglyceride Content and Glucose-3-Phosphate Dehydrogenase Activity

To determine cellular triglyceride content and glucose-3-phosphate dehydrogenase (GPDH) activity, cells were washed with cold HBSS, pH 7.4, scraped from the dishes, and collected in 50 mmol/L Trishydrochloride buffer, pH 7.4, with 1 mmol/L EDTA and 1 nmol/L mercaptoethanol. After homogenization by sonication, samples were stored at −20°C until use. Cellular triglyceride content was estimated enzymatically in cell extracts using a commercially available test kit (Boehringer, Mannheim, Germany). Activity of GPDH (EC 1.1.1.8) was used as a specific marker for differentiation. Its increase during adipose differentiation has been shown to be directly related to the increase in cellular GPDH mRNA.[29] GPDH activity was determined by spectrophotometry according to an established procedure.[30] Enzyme activity was expressed in milliunits per milligram of total cellular protein, with 1 mU being equal to the oxidation of 1 nmol NADH/min.

Determination of Glucose Uptake

To study the effects of GH on basal and insulin-stimulated glucose uptake, adipocyte precursor cells were maintained from day 1 in IT medium supplemented or not with 5 nmol/L hGH. Alternatively, adipocyte precursor cells were kept in IT medium and 5 nmol/L hGH was added after differentiation into adipocytes on day 7. On day 8, cells were washed twice with glucose-free modified Krebs-Ringer buffer with HEPES (KRH), pH 7.4, containing 1% BSA, and maintained thereafter in the same buffer for 4 hours at 37°C before glucose uptake experiments. Then 2-deoxy-D-glucose, both [^{3}H]-labeled (0.3 μCi/well) and unlabeled (final concentration, 0.1 mmol/L), was added for 5 minutes in the absence or presence of insulin (final concentration, 1 μmol/L). In other experiments, glucose uptake in cultured rat adipocyte precursor cells was found to be linear for at least 10 minutes. The incubation was terminated by washing the cells twice with ice-cold HBSS containing 200 μmol/L phloretin. Non–carrier-mediated

uptake was assessed in the presence of 40 μmol/L cytochalasin B and used for correction of raw data.

Incorporation of [^{3}H]-glucose Into Cellular Lipids

Cultures were prepared as described earlier. On day 7, cells were incubated with 0.1 μCi 3-[^{3}H]-D-glucose in basal medium (basal lipogenesis) or IT medium (insulin-stimulated lipogenesis) for 24 hours at 37°C. Then the medium was removed and cells were washed, dissolved in 1 mL 0.5N NaOH, and transferred to 25-mL plastic vials. Cellular lipids were extraced by adding 9 mL scintillation cocktail (1 L toluene containing 0.30 g 2,2′*p*-phenylen-bis-5-phenyloxazole and 5.00 g 2,5-diphenyloxazole) and counted. All values were corrected for the blanks.

Determination of Free Glycerol

Adipocyte precursor cells were maintained in IT medium with or without hGH (5 and 50 nmol/L, respectively). The medium was changed on day 4, and free glycerol was determined in culture media on day 8 using the bioluminescence method described by Kather and Wieland.[31] In other experiments, 50 nmol/L hGH was added to cells cultured in IT or ITC medium on day 7 and glycerol level was measured 4 or 24 hours later as described earlier.

GH Binding Studies

[^{125}I]-hGH binding to adipocyte precursor cells was measured on day 2, whereas [^{125}I]-hGH binding to differentiated adipocytes was measured between days 5 and 8 as indicated. Initially, cell monolayers were washed twice with 2 mL HBSS. Then, 150 pmol [^{125}I]-hGH was added in the absence or presence of increasing concentrations of unlabeled hGH, in a total volume of 0.5 mL in KRH buffer, pH 7.4, containing 0.1 mmol/L glucose and 1% BSA. The incubation was performed at room temperature (22°C for 3.5 hours and was stopped by aspirating the supernatant and rapidly washing the monolayers with 2 mL ice-cold HBSS. After solubilization of the cells in 0.5N NaOH, radioactivity of the samples was counted in a gamma counter. Nonspecific binding was measured in the presence of an excess of unlabeled hGH (0.5 μmol/L). Specific binding was obtained by subtracting nonspecific binding from total binding.

To study the downregulation of GH receptors, cells were first incubated with various concentrations of hGH for 1 or 3 hours. Then cells were washed with cold assay buffer and incubated with [^{125}I]-hGH under the same conditions as above. In parallel experiments, the monolayers were washed with an acid/salt medium (10 μL 0.1N HCl in 0.5 mL KRH buffer, pH 2.5) to remove surface-bound hGH before being exposed to [^{125}I]-hGH.[32]

Other Assays

Cellular protein content was measured according to the method of Bradford.[33] IGF-I content in culture media was determined with a radioimmunoassay for human IGF-I using a commercial kit (Nichols Institute Diagnostics, Bad Nauheim, Germany).

Statistical Analysis

Results are expressed as the mean ± SD of at least three experiments performed in duplicate. Comparisons between cell preparations maintained under various culture conditions were made using Student's *t* test.

RESULTS

Differentiation of Adipocyte Precursor Cells Into Mature Adipocytes

In serum-free medium supplemented with triiodothyronine and insulin (IT medium), up to 90% of stromal cells from rat adipose tissue differentiated into mature adipocytes. Initially, the adipocyte precursor cells had a fibroblast-like appearance and no intracellular lipids were visible by conventional microscopy (Fig 1A). During the following

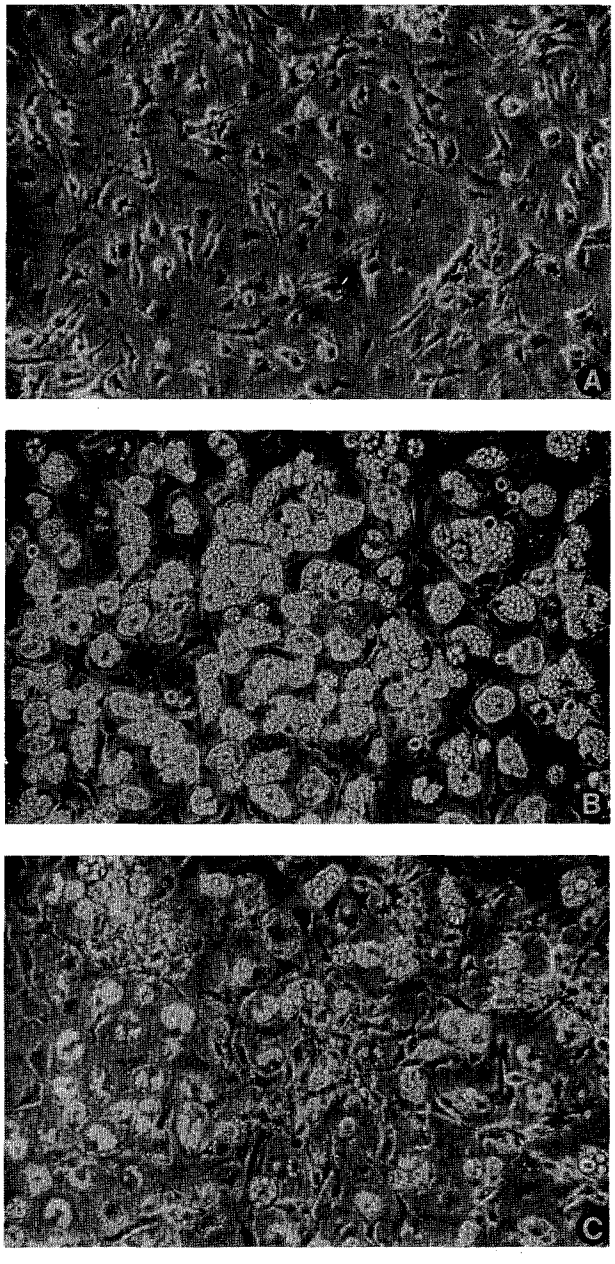

Fig 1. Microphotographs of primary cultures of rat adipocyte precursor cells and newly differentiated adipocytes (original magnification ×100). (A) Undifferentiated adipocyte precursor cells on day 2. (B) Differentiated adipocytes on day 8. Cells were cultured from day 1 to day 8 in IT medium. (C) Cells on day 8 cultured in IT medium supplemented with 5 nmol/L hGH from day 1 to day 8.

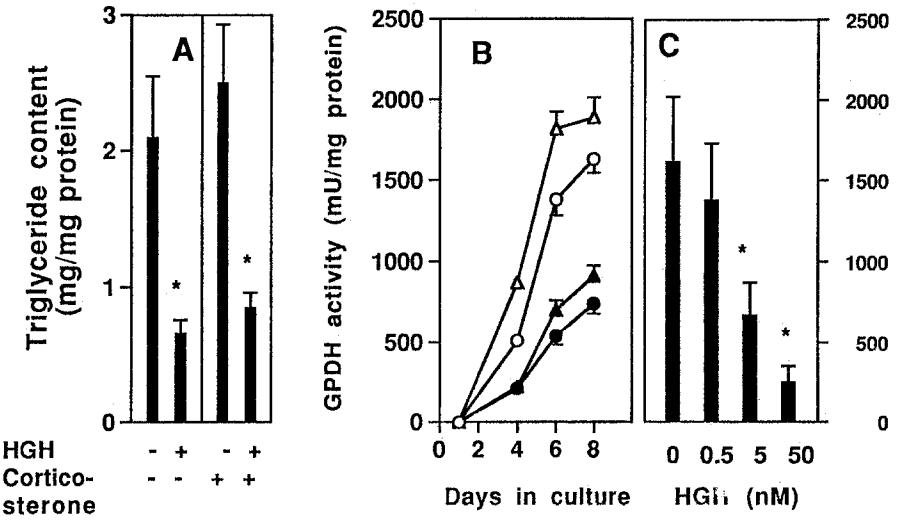

Fig 2. (A) Effect of hGH on triglyceride content of cultured rat adipocyte precursor cells. Cells were cultured in IT medium to allow differentiation. The medium was supplemented with 5 nmol/L hGH or 10 nmol/L corticosterone, or both hGH and corticosterone. On day 8, cells were harvested and triglyceride content was determined. Data are the mean ± SD of 3 experiments performed in duplicate. *$P < .01$ *v* control cells. (B) Developmental pattern of GPDH activity. Cells were cultured in IT or ITC medium as indicated. On days 1, 4, 6, and 8, duplicate cultures were harvested and GPDH activity was measured. Data are the mean ± SD of 4 separate experiments in duplicate. (○) IT medium, (●) IT medium with 5 nmol/L hGH, (△) ITC medium, (▲) ITC medium with 5 nmol/L hGH. (C) Dose-dependency of the hGH effect on GPDH activity. Cells were cultured for 8 days in IT medium with increasing concentrations (0 to 50 nmol/L) of hGH, and GPDH activity was measured on day 8. Data are the mean ± SD of 3 experiments performed in duplicate. *$P < .01$ *v* cells cultured without hGH.

days, cells rapidly acquired a round shape and accumulated lipid droplets. On day 8, most of the cells were completely filled with multiple lipid droplets (Fig 1B). Triglyceride content of the cells increased from undetectable levels on day 1 to 2.1 ± 0.5 mg/mg cellular protein in the newly differentiated adipocytes on day 8. Similarly, GPDH activity increased dramatically from undetectable values on day 1 to greater than 1,600 mU/mg protein on day 8.

Effects of hGH on Adipose Differentiation

The presence of hGH markedly diminished the differentiation of adipocyte precursor cells. When added to the cells from day 1 through day 8, hGH significantly decreased the proportion of differentiated cells on day 8 (Fig 1C) and prevented the increase in both triglyceride content (Fig 2A) and GPDH activity (Fig 2B). The inhibitory effect on GPDH activity was dose-dependent and was statistically significant at concentrations above 5 nmol/L hGH ($P < .01$; Fig 2C). The inhibitory effect of hGH on adipose differentiation was not affected by the presence of an anti–IGF-I MAb, indicating a direct action of GH (data not shown). When corticosterone was present at a concentration of 10 nmol/L, both triglyceride content and GPDH activity were slightly higher than in the absence of corticosterone, but the glucocorticoid hormone did not modulate the inhibitory action of hGH (Table 1 and Fig 2A and 2B). When newly differentiated adipocytes were exposed to 25 nmol/L hGH on day 7 for 24 hours, there was also a significant reduction of GPDH activity by 23% ± 4% as compared with control cultures ($P < .01$, data not shown).

Inhibition of adipose conversion can be exerted by at least two different mechanisms: stimulation of cell proliferation, which may lead to a decommitment of susceptible cells, and a direct antiadipogenic action unrelated to cell growth but possibly mediated by an alteration of critical metabolic pathways.

Effects of hGH on Proliferation of Adipocyte Precursor Cells

To test the first possibility, experiments on the effect of hGH on cell proliferation were performed. No increase in cell number on day 8 as compared with day 1 was observed in basal medium (Table 1). Mitogenic activity of IT medium was significantly lower than that of 10% FCS–containing medium (Table 1). Addition of hGH to the IT medium did

Table 1. Comparison of Cells of Epididymal Origin to Those From the Perirenal Region

Culture Medium	No. of Experiments	GPDH Activity (mU/mg protein)	Increase in Cell Number (%)	Specific Binding of [^{125}I]-hGH (%)
Epididymal cells				
Basal	10	ND	−19.5 ± 5.2	1.3 ± 0.1
IT	10	1,253 ± 173	89.2 ± 13.5	5.2 ± 0.2
ITC	10	1,572 ± 235*	71.2 ± 10.2	6.6 ± 0.3*
10% FCS	3	287 ± 52	427.4 ± 53.7	0.4 ± 0.0
10% NCS	3	51 ± 47	65.2 ± 22.9	0.2 ± 0.0
Perirenal cells				
IT	10	1,510 ± 213†	87.3 ± 14.2	6.5 ± 0.2†

NOTE. Epididymal and perirenal rat adipose precursor cells were cultured under serum-free culture conditions. On culture day 8, GPDH activity and [^{125}I]-hGH binding were measured. Cell number was estimated microscopically on day 1 and day 8. Data represent the mean ± SD.

Abbreviation: ND, not detected.

*$P < .01$ *v* IT medium.

†$P < .01$ *v* epididymal cells (IT medium).

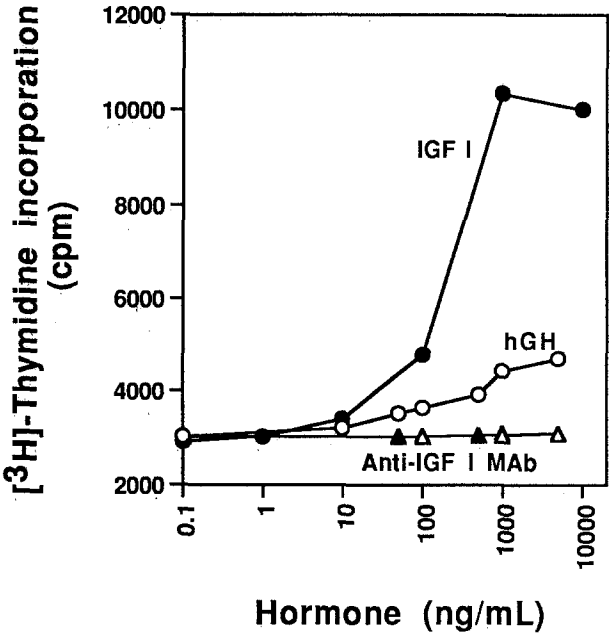

Fig 3. Effects on hGH on [³H]-thymidine incorporation by cultured rat adipocyte precursor cells in primary culture. Cells were maintained from day 1 in basal medium with or without hGH (0 to 5 mg/mL) or IGF-I (0 to 10 mg/mL). A MAb against IGF-I was added to some cultures at a 1:200 dilution. After 12 hours, [³H]-thymidine was added for another 12 hours. Then cells were harvested and cell-associated radioactivity was measured. Data are the mean of 4 experiments performed in triplicate. (○) hGH alone, (●) IGF-I alone, (△) hGH + anti–IGF-I MAb, (▲) IGF-I + anti–IGF-I MAb.

not increase either cell number or [³H]-thymidine incorporation (data not shown). In contrast, hGH dose-dependently stimulated [³H]-thymidine incorporation when cells were maintained in serum-free medium (basal medium) without other hormonal supplements (Fig 3), with the effect being significant ($P < .01$) at concentrations above 0.5 nmol/L hGH. Similarly, a slight but significant increase in cell number of approximately 30% was observed in the presence of 5 nmol/L hGH. IGF-I concentration in media of cells exposed to 5 nmol/L for 48 hours was significantly higher than in media of unexposed cells (28.1 ± 5.9 *v* 2.3 ± 0.6 ng/mL, $P < .01$). Similar to hGH, addition of IGF-I also dose-dependently stimulated [³H]-thymidine incorporation (Fig 3). Addition of a mouse anti–IGF-I MAb completely inhibited [³H]-thymidine incorporation induced by both hGH and IGF-I (Fig 3), indicating that mitogenic activity of hGH was mediated by increased synthesis of IGF-I.

Effects of hGH on Glucose Uptake and Lipogenesis

In the culture system used, glucose uptake is a rate-limiting step for lipid synthesis and accumulation, since the serum-free medium was devoid of exogenous lipids. Therefore, we performed studies on glucose transport and incorporation into extractable lipids in the presence and absence of hGH. Basal uptake of [³H]-2-deoxy-D-glucose markedly increased during adipose differentiation from 0.01 pmol/dish on day 1 to 0.17 pmol/dish on day 8. Insulin-stimulated glucose transport increased in a similar pattern during differentiation from 0.02 pmol/dish on day 1 to 0.24 pmol/dish on day 8. Exposure of cells to 5 nmol/L hGH for the whole culture period resulted in a reduced increase in basal (data not shown) and insulin-stimulated glucose uptake (Fig 4A). When hGH at 25 nmol/L was added on day 7 for 24 hours to differentiated adipocytes, there was also a significant reduction in glucose uptake (Fig 4A). The presence of corticosterone in the culture medium had no significant effect on glucose uptake independently of the presence of hGH.

Continuous exposure of the cells to 5 nmol/L hGH in IT medium resulted in a dramatic decrease of insulin-stimulated lipogenesis on day 8 (Fig 4B). Again, corticosterone at 10 nmol/L did not significantly affect the inhibitory effect of hGH on lipogenesis (Fig 4B). There was also an inhibitory effect of hGH on lipogenesis in newly differentiated adipocytes (Fig 4B). Similar results were obtained in the absence of insulin (data not shown).

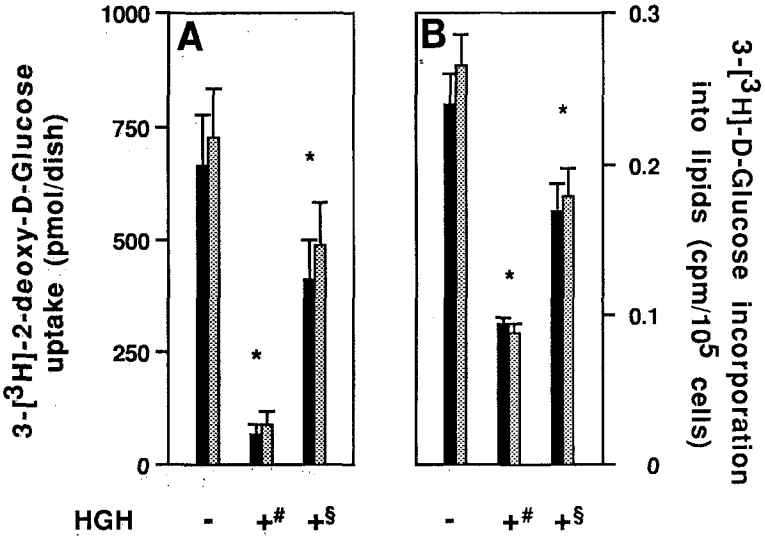

Fig 4. Effects of hGH on insulin-stimlulated glucose uptake and lipogenesis. Cells were cultured in either IT medium (■) or ITC (▨) as indicated. 5 nmol/L hGH was added between day 1 and day 8 to differentiating adipocyte precursor cells (#) or on day 7 to newly differentiated adipocytes (§). (A) [³H]-2-deoxy-D-glucose uptake determined on day 8. Data are the mean ± SD of 3 experiments in duplicate. (B) Incorporation of 3-[³H]-D-glucose into cellular lipids over 24 hours. Data are the mean ± SD of 3 experiments performed in duplicate. *$P < .01$ *v* cells cultured without hGH in IT or ITC medium, respectively.

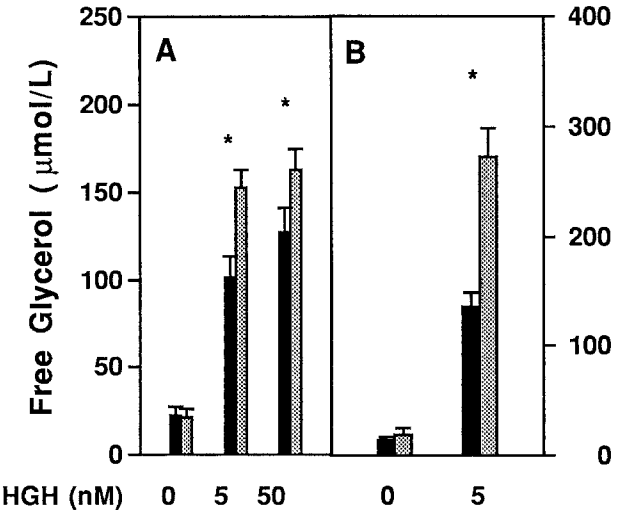

Fig 5. Effects of hGH on glycerol release. (A) hGH (5 and 50 nmol/L, respectively) was added from day 1 to cells kept in IT medium (■) or ITC medium (▨). On days 4, the culture medium was changed. Glycerol concentration of the culture medium was measured on day 8. (B) hGH was added on day 7 to newly differentiated adipocytes kept in IT medium (■) or ITC medium (▨), and glycerol content was measured 24 hours later (day 8). Data are the mean ± SD of 3 experiments performed in duplicate. *$P < .01$ v control cells.

Effects of hGH on Lipolysis

Another mechanism by which hGH could reduce cellular triglyceride content is by stimulating lipolysis. To study the effect of hGH on lipolysis, release of glycerol into the culture medium was measured under various conditions. In cells exposed to 5 and 50 nmol/L hGH, respectively, from day 1 to day 8, the amount of glycerol in the culture media on day 8 was significantly higher than in cells cultured in the absence of hGH (Fig 5). hGH-induced glycerol release was higher in the presence than in the absence of corticosterone ($P < .01$; Fig 5A), which by itself had no effect on lipolysis (data not shown). When hGH was added to differentiated cells, a clear increase in lipolysis was seen after 24 hours, which was again more pronounced in the presence of corticosterone ($P < .01$; Fig 5B). Similar lipolytic activity of hGH was obtained when insulin and triiodothyronine were omitted from the medium (data not shown).

Binding of [^{125}I]-hGH to Undifferentiated and Differentiated Cells

Binding of [^{125}I]-hGH to cultured adipocytes was both temperature- and time-dependent. Maximum binding was obtained with an incubation time of 3.5 hours at 22°C. Measurement of [^{125}I]-hGH binding during differentiation showed a significant increase in total binding from day 2 to day 7 (Fig 6). This increase was clearly more pronounced in cells cultured in IT medium, where most of the cells underwent adipose conversion, than in cells cultured in basal medium, where adipose conversion was not detectable ($P < .01$; Fig 6). Interestingly, corticosterone increased specific binding of [^{125}I]-hGH in a dose-dependent manner (Table 1). [^{125}I]-hGH binding in cells grown in the presence of 10% FCS or 10% NCS was significantly lower than in cells cultured in either basal or IT medium (Table 1 and Fig 6), probably due to the downregulation of receptors by GH present in serum.

Preincubation of the cells for 1 or 3 hours with various concentrations of unlabeled hGH resulted in a decrease in their ability to subsequently bind [^{125}I]-hGH by up to 60%, showing that downregulation occurs rapidly. Downregulation was also observed when cells were first washed with an acid/salt medium to remove surface-bound unlabeled hGH before being exposed to [^{125}I]-hGH. The degree of downregulation was greater after 3 hours than after 1 hour of preincubation with unlabeled hGH (data not shown).

A comparison of total [^{125}I]-hGH binding to cultured cells from the two different depots demonstrated a significantly higher binding in adipocyte precursor cells from perirenal adipose tissue as compared with the epididymal depot (Table 1).

Competition experiments were performed on days 2 and 6, respectively, by incubating cells with 150 pmol/L [^{125}I]-hGH and increasing concentrations (0 to 500 nmol/L) of unlabeled hGH (Fig 7). Scatchard analysis of the data yielded a linear plot (Fig 7, insert) with apparent (K_ds) of 0.14 and 0.16 nmol/L, respectively, on day 2 and day 6. Estimated numbers of GH binding sites were 3,000 and 8,400, respectively, indicating an increase of GH receptors during adipose differentiation.

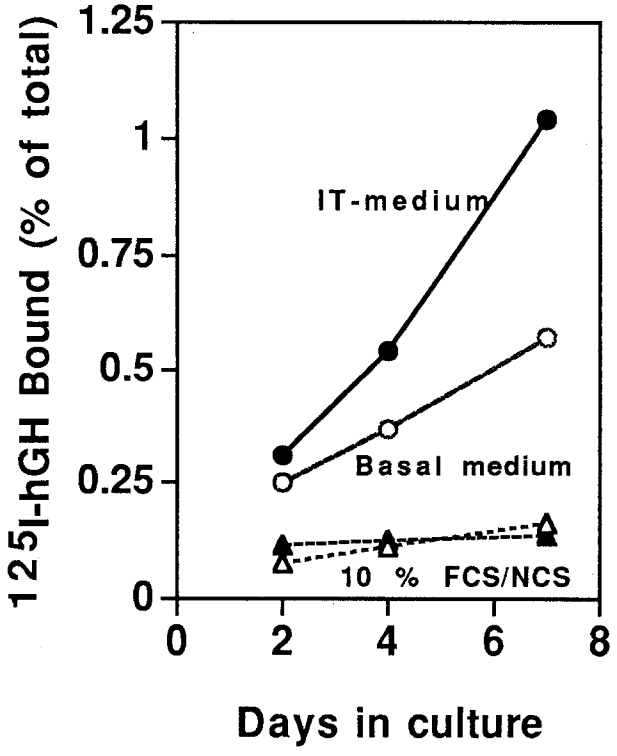

Fig 6. [^{125}I]-hGH binding of cultured rat adipocyte precursor cells during differentiation. [^{125}I]-hGH binding to rat adipocyte precursor cells was measured after 2, 4, and 7 days. Results are expressed as a percentage of added radioactivity per 100,000 cells (specific binding). Data are the mean of triplicate samples of 1 experiment, representative of 3 others. (●) IT medium, (○) basal medium, (△) basal medium supplemented with 10% FCS, (▲) basal medium supplemented with 10% NCS.

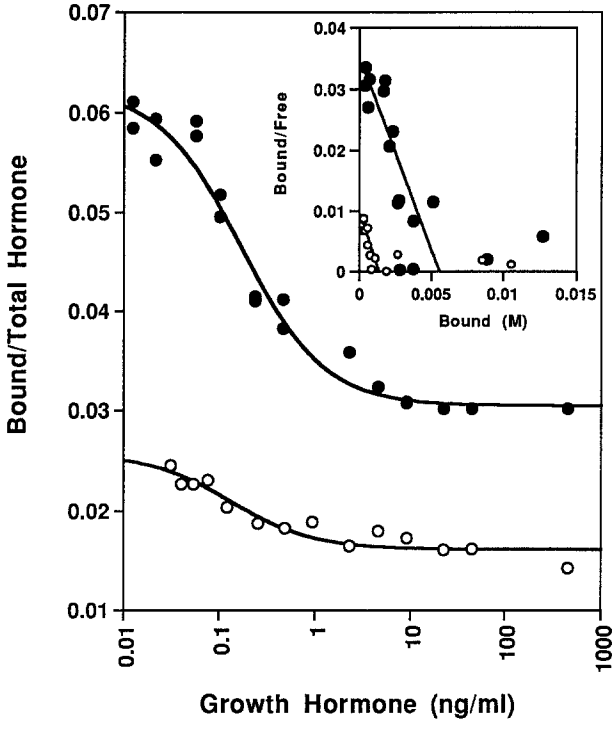

Fig 7. Inhibition of [^{125}I]-hGH binding by unlabeled hGH in cultured rat adipocyte precursor cells before and after differentiation. [^{125}I]-hGH binding was determined on day 2 (○) and day 6 (●) in KRH buffer for 3.5 hours at room temperature (22°C) at increasing concentrations (0 to 0.5 μmol/L) of unlabeled hGH. [^{125}I]-hGH binding is expressed as the ratio bound:total hormone concentration and represented as a function of unlabeled hGH concentration. Insert: The same data (days 2 and 6) represented in Scatchard coordinates (bound/free *v* bound).

DISCUSSION

The effect of GH on adipose differentiation has been almost exclusively studied in clonal preadipocyte cell lines. These studies strongly suggested a stimulatory action of GH on fat cell formation.[5-8] In contrast, limited information is available on the action of GH on the differentiation of adipocyte precursor cells in primary culture. Previous preliminary studies performed with cells from rat and human adipose tissue failed to demonstrate a similar stimulation by GH, but also did not show an inhibitory effect, in contrast to our present results.[22,23] The most likely explanation for this discrepancy is that these experiments were performed in serum-containing media. Under these conditions, downregulation of GH receptors and the incalculable effect of other serum compounds on proliferation and differentiation may have diminished or concealed possible effects of GH. To avoid such interference, we used a serum-free chemically defined culture system to examine the action of GH on adipose differentiation of cultured rat adipocyte precursor cells.

When cells were triggered to differentiate by insulin and triiodothyronine, GH significantly diminished the increase in GPDH activity and markedly reduced the proportion of newly formed adipocytes, as well as the lipid content, of the cultures. These results are in agreement with those recently reported by Hausman and Martin,[34] who showed a GH-induced reduction of approximately 50% in both the size and number of fat cell clusters in cultured pig adipose precursor cells. This different pattern of GH action on the differentiation of primary adipocyte precursor cells and clonal preadipocyte cell lines points to a substantial biological difference between the two cell models. There is now growing evidence that the two models behave differently to some degree and may represent different stages of the adipocyte lineage.

The exact mechanism by which GH exerts its adipogenic effect in preadipocyte cell lines is far from being understood. It has been shown that GH directly activates protein kinase C, thereby leading to a rapid and transient stimulation of c-*fos* gene transcription.[35] However, part of the GH effect could also be attributed to IGF-I, since GH has been shown both to induce IGF-I gene expression[36] and to increase sensitivity of the cells to the action of IGF-I.[37] IGF-I, in turn, has been reported to promote adipose differentiation of clonal cell lines.[29,38] Although GH markedly stimulated IGF-I production in rat adipose precursor cells as well, the net result of the GH effect was a diminution of adipose differentiation. Concomitantly, GH exhibited a growth-promoting activity. Addition of an anti–IGF-I MAb prevented the stimulatory effect on cell proliferation but not the reduction of differentiation, indicating that the antiadipogenic action of GH was unrelated to growth promotion and was not mediated by IGF-I.

The finding of a proliferation-stimulating effect of GH in cultured rat adipocyte precursor cells is in contrast to results reported in a recent study in 3T3-F442A cells. In this cell line, GH induced a state of growth arrest that may be a prerequisite for GH-induced adipose differentiation.[39] This substantial difference between the two models provides further evidence that mechanisms active in clonal cell lines are not necessarily the same as those occurring during adipose differentiation of adipose tissue–derived stromal cells in primary culture.

It is obvious from our experiments that the antiadipogenic activity of hGH could be due to its disturbance of glucose and lipid metabolism. hGH not only potently reduced glucose transport and thereby lipogenesis from glucose, but also exhibited a lipolytic action. Both effects may explain the reduced lipid content of the cells in the presence of hGH. These effects were not restricted to the differentiation phase, but were also demonstrable after acquisition of the adipocyte phenotype. These findings are in agreement with earlier reports on the metabolic effects of GH in 3T3-F442A adipocytes[15] and freshly isolated adipocytes.[4,13] It is poorly understood at present how GH interferes with glucose uptake in fat cells. However, experiments in 3T3-F442A cells suggest that GH decreases the synthesis of glucose transporter-1 (GLUT-1), whereas the amount of GLUT-4 seems to remain stable.[40]

The effects of hGH on adipose conversion, as well as on fat cell metabolism, were also investigated in the presence of corticosterone. One recent study has shown that both hormones have opposing effects on adipose differentiation of porcine adipocyte precursor cells.[26] An explanation for these observations could not be provided. Our data show

that the stimulatory effect of corticosterone on GPDH activity, as well as on glucose uptake and lipogenesis, was independent of the absence or presence of hGH, indicating that the effects of hGH and corticosterone may be due to different intracellular mechanisms. Earlier studies have shown that the GH-induced lipolytic response in isolated rat fat cells is amplified by glucocorticoids.[4] Our data suggest that the potentiating effect of corticosterone on lipolysis is at least partly due to an increase in the number of hGH receptors, since glucocorticoids increased [^{125}I]-hGH binding.

Our binding experiments indicated an average receptor number of 8,400/cell in differentiated cells. This figure is comparable to that previously reported in freshly isolated adipocytes.[14,19] However, the observed increase in hGH binding in differentiating adipocyte precursor cells is in contrast to the findings in clonal preadipocyte cell lines, in which GH binding was almost unchanged independently of the differentiation state.[20,21] It is interesting that adipocyte precursor cells from perirenal adipose tissue not only showed higher GPDH activities in response to adipogenic factors but also demonstrated a higher specific binding of hGH as compared with cultures from epididymal adipose tissue. This finding points to a depot-specific variation in the binding and action of GH in adipose tissue.

In conclusion, this study clearly demonstrates a variety of GH-induced effects in both rat adipocyte precursor cells and newly developed fat cells. These actions generally aim at reducing the lipid content of adipocytes, thereby limiting adipose tissue size. On the other hand, GH seems to contribute to an enlargement of the pool of adipocyte precursor cells by inducing an increased local production and secretion of IGF-I. The culture system used here may represent a valuable tool to gain a better understanding of the physiological role of GH in the regulation of adipose tissue growth and metabolism.

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