Promoting Effect of Glucocorticoids on the Differentiation of Human Adipocyte Precursor Cells Cultured in a Chemically Defined Medium

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Abstract

Stromal-vascular cells obtained from adult human subcutaneous adipose tissue were cultured in a chemically defined serum-free medium. In the presence of 0.2 nM triiodothyronine and 0.5 μM insulin, up to 25% of the cells were able to undergo terminal adipose differentiation within 18 d, as assessed by lipid accumulation and the expression of lipoprotein lipase (LPL) and glycerol-3-phosphate dehydrogenase (GPDH) activities. Addition of cortisol resulted in a potent dose-dependent stimulation of the adipose differentiation process. Cortisol could be replaced by dexamethasone and partly by aldosterone, but not by sex steroids. The proportion of differentiated cells was dependent upon the age of the donor; when isolated from young adults, up to 70% of the stromal-vascular cells expressed the adipocyte phenotype as compared with 5–10% when the cells were isolated from the oldest subjects. An inverse relationship was observed between the age of the 27 normal-weight donors and the extent of GPDH expression after maintenance of the cells for 18 d in chemically defined medium supplemented with insulin, triiodothyronine, and cortisol ($r = -0.787$, $P < 0.001$). It is concluded that adult human adipose tissue still contains precursor cells that are able to undergo adipose differentiation in vitro. This improved culture system may offer the opportunity to characterize other adipogenic factors as well as antiadipogenic factors involved in the control of adipose tissue growth.

Introduction

The present knowledge regarding the cellularity of adipose tissue in man is mainly derived from fat cell sizing studies. Previous investigations of the adipose tissue cellularity have shown that both the number and size of existing fat cells contribute to the adipose tissue mass. It was originally believed that the number of fat cells is fixed during childhood and that childhood-onset obesity is associated with adipocyte hyperplasia, whereas adult-onset obesity was postulated to be characterized by fat cell enlargement only (1, 2). This concept was challenged by later studies showing that the formation of new fat cells can also occur in adult life. The degree of hyperplasia appeared to be well correlated with the severity of overweight independent of the time of onset (3). However, up to now, the characteristics of the cellular development of human adipose tissue, including that of overweight patients, are far from being understood.

It is now well established that fibroblast-like adipose precursor cells, capable of undergoing differentiation into adipose cells, are present in the adipose tissue of various species including man. The existence of these cells has been originally suggested by studies after the incorporation of radiolabeled thymidine into DNA of developing rat adipose tissues (4–6). Both histological studies (7) and studies with cultured stromal-vascular cells isolated from adipose tissue (8–11) provided evidence for the existence of specific adipocyte precursor cells in man. However, in the various attempts using culture conditions developed for animal models and clonal cell lines, a very limited number of human stromal-vascular cells, if any, was found to develop the characteristic adipocyte phenotypes.

We recently reported that the adipose conversion of cultured stromal-vascular cells from adipose tissue samples of adult humans in serum-containing medium was enhanced when the medium was supplemented with insulin, cortisol, and 1-*methyl*-3-isobutylxanthine (MIX),[1] the latter compound being present for the first 3 d after confluence. Under these conditions, up to 5% of the cells developed biochemical and morphological characteristics of mature adipocytes (12). The differentiation of human adipose precursor cells was also demonstrated using a serum-free chemically defined medium. In the presence of supraphysiological concentrations of insulin, up to 20% of the cells accumulated lipid droplets and expressed lipoprotein lipase and glycerol-3-phosphate dehydrogenase (GPDH) activities (13). As shown in the present report, it is now possible to define conditions under which up to 70% of the stromal-vascular cells isolated from adipose tissue samples of young adults are able to undergo adipose conversion. With this serum-free medium containing insulin, triiodothyronine and glucocorticoids, the frequency of adipose conversion was strongly decreased as a function of the age of the donor.

Methods

Subjects. Adipose tissue samples were obtained from the subcutaneous abdominal depot of 17 male and 10 female adults in the age range between 20 and 83 yr undergoing elective abdominal surgery. Patients suffering from an inflammatory or malignant disease were excluded. The operations were carried out for the following reasons: 10 for herniotomy, 7 for cholecystectomy, 3 for abdominal vascular surgery, 3

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1. *Abbreviations used in this paper:* GPDH, glycerol-3-phosphate dehydrogenase (EC 1.1.1.8); LPL, lipoprotein lipase (EC 3.1.1.34); MIX, 1-methyl-3-isobutylxanthine.

for transabdominal colon or Sigma polypectomy, 2 for selective proximal vagotomy, and 1 for tubal ligation. All subjects were of normal weight as defined as a body mass index under 27.8 kg/m^2 for male and 27.3 kg/m^2 for females (14). All patients had a normal physical examination and routine laboratory tests were within normal limits. None of the subjects was under a reduction diet at the time of examination. The data on age and body mass index of the individual subjects are given in Table I. The procedure followed in this investigation has been approved by the Ethical Committee of the University of Ulm.

Isolation and culture of stromal-vascular cells. The isolation of stromal-vascular cells was performed according to the method originally described by Björntorp et al. (15) with some modifications. Tissue samples (2–15 g) from the individual donors were kept at room temperature in PBS containing 20 mg/ml BSA (Sigma Chemical Co., Munich, West Germany), pH 7.4, and immediately transferred to the laboratory. The samples were repeatedly rinsed in PBS to remove any blood. Fibrous material and blood vessels were carefully dissected and discarded. The remaining tissue cut into small pieces (10–20 mg) was digested in Krebs-Ringer buffered with 25 mM Hepes containing 1.5 mg/ml collagenase (CLS type I; Worthington Biochemical Corp., Freehold, NJ) and 20 mg/ml BSA in a two-step procedure. The ratio between adipose tissue mass to incubation solution was 1 g/4 ml. The first digestion was carried out for 30–45 min at 37°C under intermittent shaking. The dispersed tissue was filtered through a nylon mesh (pore size 250 μm). The remaining tissue was again digested for another 30 min and then filtered as above. More than 95% of the tissue was disaggregated by this procedure and the remaining fibrous material was discarded. Both cell suspensions were mixed and centrifuged for 10 min at 200 *g*. The main contaminating cells of the cell suspension were erythrocytes, which constituted more than 90% of total cell number at this stage. The high contamination with red blood cells was found to markedly decrease cell adherence and proliferation. To eliminate red blood cells, the stromal-vascular cell fraction was incubated with a erythrocyte lysing buffer consisting of 0.154 M NH_4Cl, 10 mM $KHCO_3$, and 0.1 mM EDTA for 10 min at room temperature. Under these conditions, > 95% of red blood cells were lysed without damaging the nucleus-containing cells as assessed by Trypan blue exclusion. The application of the erythrocyte lysing buffer did not interfere with cell attachment, growth or differentiation of nucleus-containing cells in the stromal-vascular fraction. (Control experiments showed that treatment with the erythrocyte lysing buffer reduced dramatically the number of red blood cells previously mixed with 3T3-L1 cells, with no change in growth and differentiation of the latter.) After additional washing and centrifugation steps, the floating mature adipocytes were aspirated and the sedimented stromal-vascular fraction resuspended in DME/Ham's F-12 medium (1:1, vol/vol) supplemented with 10% FCS (Seromed, Berlin, West Germany), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Boehringer, Mannheim, West Germany). Aliquots of the cell suspension were counted with a hemocytometer to determine the number of nucleus-containing cells. Cells were inoculated at various densities as indicated into 12-well plates, each well representing ~ 4.5 cm^2 (Linbro, Flow Laboratories, McLean, VA). After 16–20 h routinely used for cell attachment, cells were carefully washed with PBS to remove nonadhering material, mainly white blood cells and cell debris. Unless otherwise stated cells were inoculated at a density of 30,000/cm^2. Under these conditions, cultures were usually in a preconfluent stage after the 20-h period of cell attachment. Cells were then refed with a chemically defined serum-free medium consisting of DME/Ham's F-12 medium (1:1, vol/vol), 15 mM $NaHCO_3$, 15 mM Hepes, 33 μM biotin, 17 μM pantothenate, 0.5 μM human insulin (kindly provided by Hoechst, Frankfurt, West Germany), 0.2 nM triiodothyronine, and antibiotics as described previously (13). This medium is referred to as ITT medium (13). Cells were subsequently maintained in this medium supplemented as indicated in the legends of Tables and Figures. Cultures were refed every 2–3 d. Human skin fibroblasts kindly provided by Dr. J. Ittner (University of Ulm, West Germany) and foreskin fibroblasts kindly provided by Dr. F. Bastiani (University of Nice, France) were also used in this study.

Table I. Characteristics of the 27 Subjects

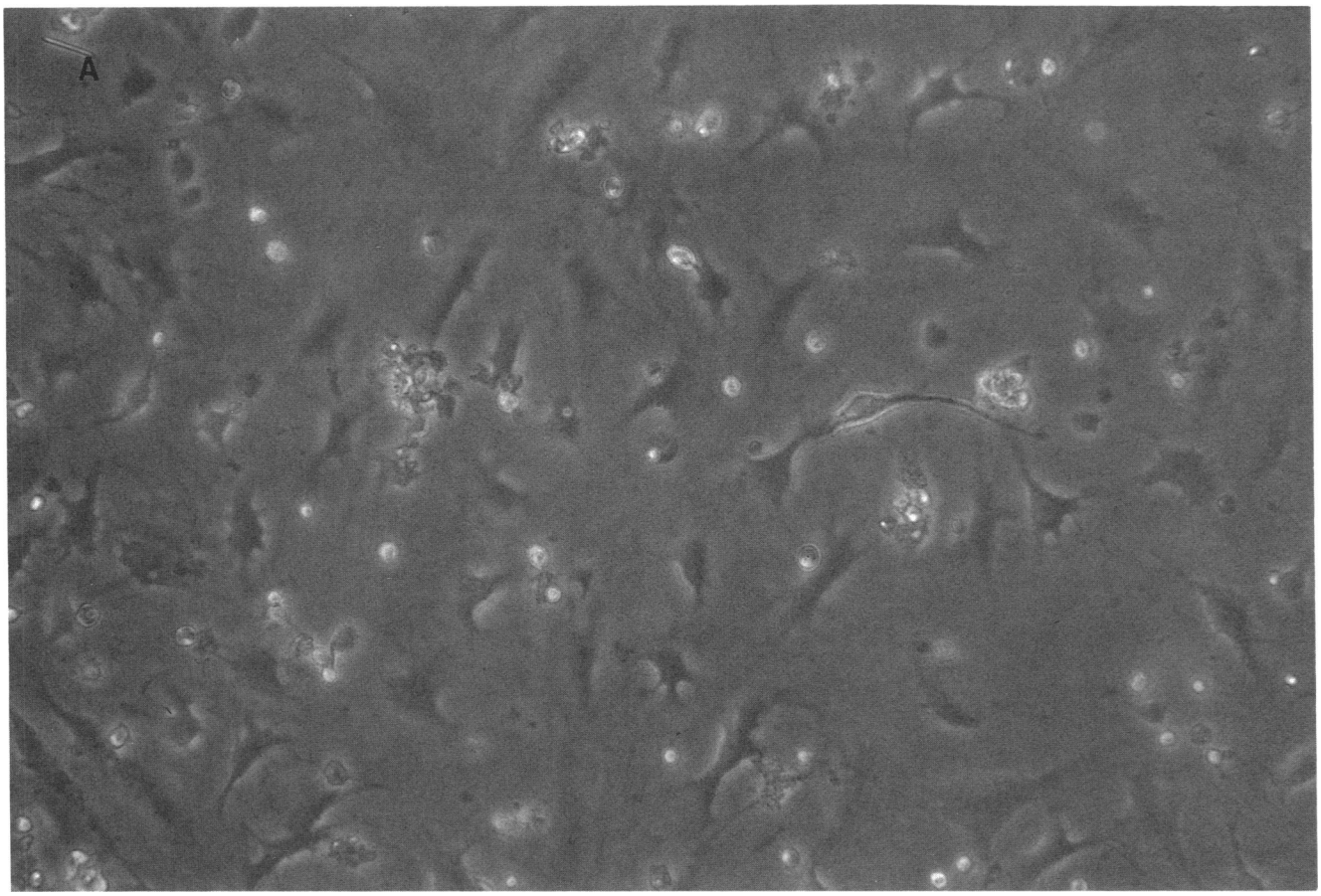
No.	Sex	Age	Body mass index	GPDH activity
			kg/m^2	*mU/mg protein*
1	M	38	27.1	1163
2	M	22	27.7	1413
3	M	47	24.0	530
4	M	47	21.3	880
5	M	20	22.7	760
6	M	58	23.6	256
7	M	20	21.6	1068
8	M	21	22.7	1006
9	M	67	24.8	145
10	M	65	27.0	196
11	M	24	24.5	1636
12	M	42	27.4	932
13	M	22	21.7	1151
14	M	63	25.9	150
15	M	52	26.7	337
16	M	20	26.7	868
17	M	82	25.5	235
18	F	48	20.7	252
19	F	35	22.7	1162
20	F	66	26.4	332
21	F	45	27.0	519
22	F	49	23.8	310
23	F	83	24.0	288
24	F	22	24.4	593
25	F	45	24.2	473
26	F	54	26.6	296
27	F	21	22.1	1086

Cultures of stromal-vascular cells were obtained from the adipose tissue samples of the donors inoculated and cultured under standardized conditions as described in Methods (duplicate dishes). After culture for 18 d in ITT medium supplemented with 0.1 μM cortisol, cells were harvested for the determination of GPDH activity.

Cells were regarded as differentiated by morphological criteria when, after acquiring a round shape, their cytoplasm was completely filled with multiple lipid droplets (also assessed by Oil Red O staining). The proportion of differentiated cells was estimated in some experiments by direct counting under the microscope total and differentiated cells present as a monolayer, using a micrometer (Zeiss Oberkochen, West Germany) at a 100-fold magnification.

Factor VIII antigen immunofluorescence labeling. After elimination of red blood cells, endothelial cells represent the main contaminant of the stromal-vascular fraction. However, this cell type can be easily detected by its cobblestone appearance and is characterized by the presence of Factor VIII antigen. The number of endothelial cells was estimated by means of immunolabeling of Factor VIII antigen (16). Briefly, cells were seeded at 20,000 cells/cm^2, grown to confluence on glass slides in 4-well chamber systems (Miles Scientific, Naperville,

Figure 1. Morphology of differentiating stromal-vascular cells from human adipose tissue. After seeding in serum-containing medium (for 16 h), cells were cultured in ITT medium supplemented with 0.1 μM cortisol for 18 d. (*A*) Cells on day 0 and (*B*) cells on day 18 before harvesting. ×100.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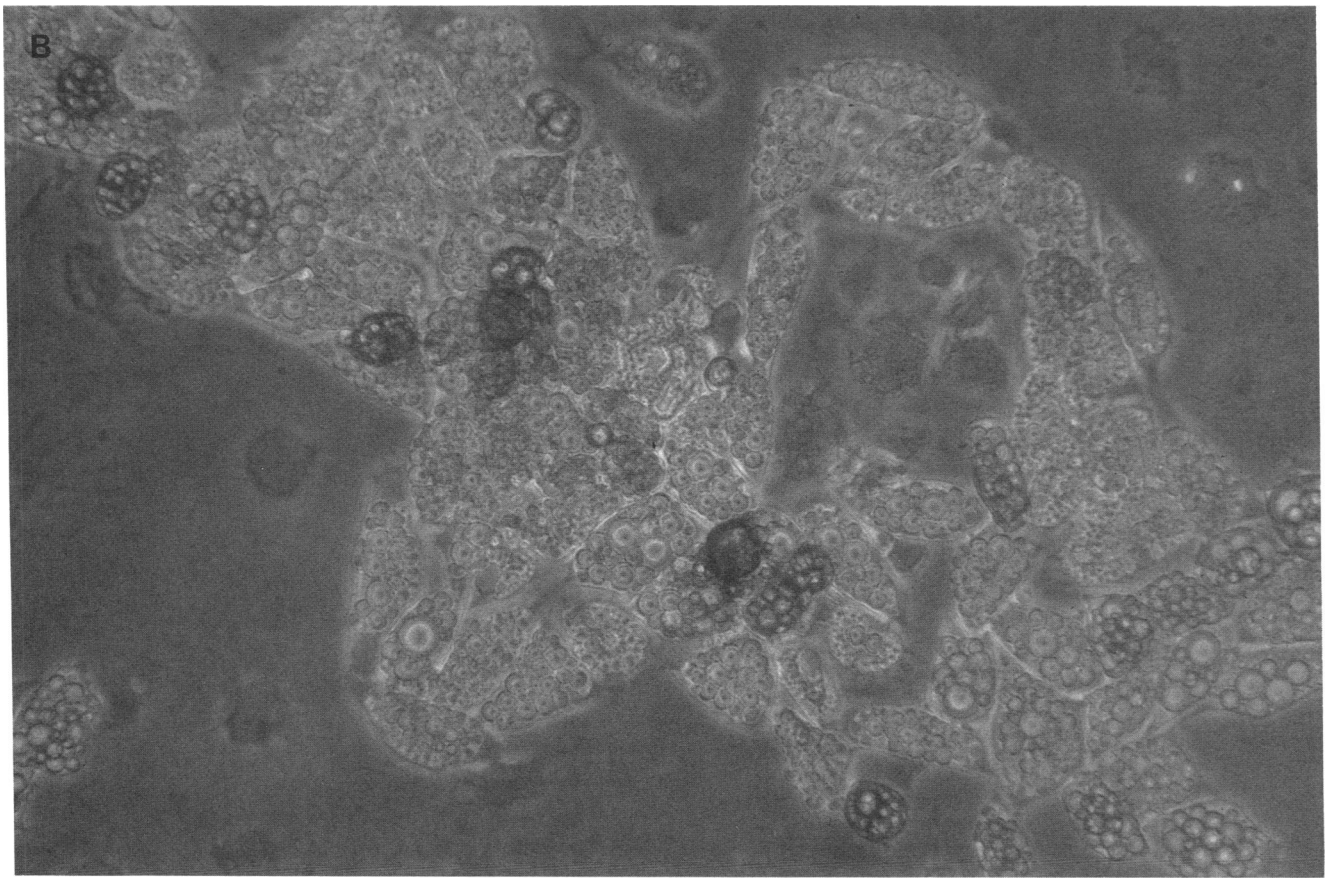
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IL), rinsed with PBS (pH 7.2) and fixed in acetone for 10 min at 0°C. Cultures were then incubated in a moist chamber at 37°C for 30 min first with rabbit anti-serum Factor VIII (Dianova, Hamburg, West Germany) or nonimmune rabbit IgG (Dianova) and second, after thorough rinsing in PBS, with fluorescein-conjugated anti-rabbit IgG (Dianova). The number of fluorescent cells was counted by fluorescence microscopy (Zeiss).

Enzymatic and chemical determinations. After 18 d of maintenance under culture conditions as indicated, cells were washed with PBS (pH 7.4), harvested in prechilled 25 mM Tris-HCl buffer containing 1 mM EDTA (pH 7.5), and homogenized using a Branson sonifier (Branson Sonic Power Co., Danbury, CT). GPDH activity was determined spectrophotometrically in the sonicated cell extracts (17). The assay mixture contained 100 mM triethanolamine HCl buffer (pH 7.5), 2.5 mM EDTA, 0.12 mM NADH, 0.1 mM mercaptoethanol, and to start the reaction, 0.2 mM dihydroxyacetone phosphate. Results are expressed as mU/mg protein, 1 mU being equal to the oxidation of 1 nmol NADH/min.

For the determination of lipoprotein lipase (LPL) activity, cells suspended in cold 50 mM NH_3/NH_4Cl buffer (pH 8.1) (0.4 ml per dish) containing 3 U/ml heparin were sonicated and centrifuged at 500 *g* for 5 min. LPL activity was measured using suitable amounts of the resulting supernatant fraction and a serum-activated emulsion of tri[9,10-^{3}H]oleylglycerol (Amersham-Buchler, Braunschweig, West Germany) stabilized with phosphatidylcholine (Sigma Chemical Co., St. Louis, MO) as described earlier (18). 1 mU of LPL activity represented the serum-dependent release of 1 nmol of fatty acid/min. A protein precipitation technique using sodium deoxycholate and TCA was applied to measure protein contents without interfering lipids (19). The triacylglycerol content of the cultures was measured using a commercially available test kit (Boehringer).

Results are expressed as means±SE. Correlation coefficients were calculated using linear regression analysis.

Results

Stromal-vascular cells were obtained by a two-step collagenase digestion of adipose tissue samples and initially maintained for 16–20 h in serum-containing medium to allow cell attachment. At that time, cells were in a preconfluent stage and exhibited a fibroblast-like morphology (Fig. 1 *A*). Cells were completely devoid of lipid droplets as assessed by Oil Red O staining and measurement of triacylglycerol content. The cultures of stromal-vascular cells were essentially free of endothelial cells as assessed by direct examination under the microscope. Furthermore, immunofluorescence labeling using a specific antiserum directed against Factor VIII antigen indicated that the percentage of endothelial cells was usually < 1%. Cells were subsequently exposed to ITT medium supplemented with 0.1 μM cortisol. The cells started to accumulate small refringent lipid droplets within 8 d and were able 4–12 d later to develop an adipocyte-like morphology, the cytoplasm being filled with triacylglycerol droplets (Fig. 1 *B*), which stained readily with Oil Red O. Under these conditions, up to 70% of the stromal-vascular cells from young adults were filled with triacylglycerol droplets after 18 d of culture. It must be recalled that a good correlation between the proportion of differentiated triacylglycerol-containing cells and the specific activity of GPDH occurred both for human stromal-vascular cells (12) and mouse Ob17 cells (20). Furthermore, histochemical staining of GPDH was only observed in clusters of lipid-containing cells (13). In agreement with the number of lipid-filled cells detected microscopically, the GPDH activities remained below the detection limit from day 0 to day 4, became measurable at day 8 and sharply increased, reaching maximum levels at day 16 (Fig. 2). LPL, the activity of which was undetectable at day 0, was expressed at a significant level (7 mU/mg, i.e., 40% of the maximal activity) as early as day 4, reached a maximal level at days 8–12 and decreased thereafter (Fig. 2). Thus it appears that the emergence of LPL precedes that of GPDH. It is interesting to note that adult human skin fibroblasts as well as infant foreskin fibroblasts cultured under identical conditions neither exhibit changes in cell morphology nor express LPL and GPDH activities nor accumulate lipid droplets (data not shown and reference 13).

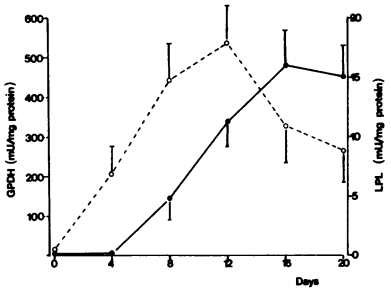

Figure 2. Time-course of the development of LPL and GPDH activities in cultured stromal-vascular cells from human adipose tissue. Cells inoculated as described in Methods were subsequently cultured for 20 d in ITT medium supplemented with 0.1 μM cortisol. Duplicate cultures were harvested every 4 d from day 0 for the determination of LPL (○) and GPDH (●) activities (mean±range of two experiments).

The extent of adipose conversion was strongly dependent upon the inoculation density. As demonstrated in Fig. 3, the highest GPDH activity values were obtained at a seeding density range of 30,000 to 40,000 cells/cm^2. The time of exposure to serum-containing medium before exposure to serum-free medium appeared also to be important: the GPDH activity was maximal when cells were changed to the serum-free medium 4 h after inoculation, which was a time period sufficient to allow cell attachment (Table II). In contrast, exposure to serum-containing medium for 90–180 h led to a significant decrease of the GPDH activity by ~ 70% and 85%, respectively. This loss of differentiation capacity was accompanied by a significant increase in cellular protein content, reflecting a sustained proliferation visible by microscope examination. After maintainance in serum-free medium of confluent cells previously cultured in the presence of serum, a nearly complete loss of differentiation capacity was observed (Table II).

Consideration was next given to the role of glucocorticoids. In ITT medium, the adipose conversion of stromal-vascular cells of infants (13) or adults (see Fig. 5) did not exceed 20% of the cell population and the corresponding GPDH activity values were in the range of 50 to 400 mU/mg protein; the addition of glucocorticoids considerably enhanced the percentage of differentiated cells and the GPDH activity. This effect of glucocorticoids was dependent upon the presence of insulin, since adipose conversion was hardly detectable in its absence: under these conditions, no lipid accumulation was observed and the GPDH activity values remained in the range of 30 to 70 mU/mg protein. The number of differentiated cells was dependent upon the glucocorticoid concentration. In combination with 0.5 μM insulin, cortisol was able to enhance the adipose conversion in a dose-dependent manner as illustrated by the increase in GPDH specific activity (Fig. 4). Its effect was already significant at 1 nM whereas the GPDH activity was 4.2-fold above the basal level at 1 μM cortisol. Cor-

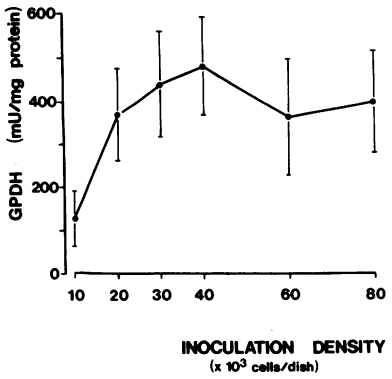

Figure 3. Dependence of GPDH activity on the inoculation density of cultured stromal-vascular cells from human adipose tissue. Cells were seeded at the densities indicated and maintained for 16 h in serum-supplemented medium. They were subsequently fed with ITT medium supplemented with 0.1 μM cortisol. After 18 d in culture, cells were harvested for the determination of GPDH activity (mean±range of two separate experiments performed with duplicate dishes).

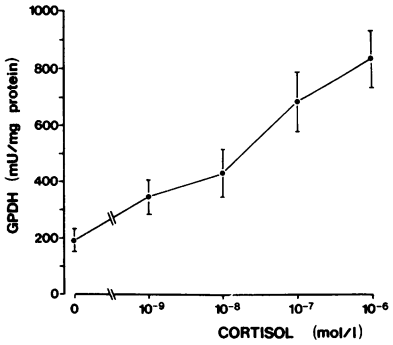

Figure 4. Dose-response relationship of cortisol to GPDH activity in cultured stromal-vascular cells from human adipose tissue. After inoculation in serum-containing medium for 16 h, cells were maintained for 18 d in ITT medium supplemented or not with increasing concentrations of cortisol as indicated. Cells were then harvested for the determination of GPDH activity. Mean±SE of three separate experiments performed on duplicate dishes.

tisol could be replaced by dexamethasone and partly by aldosterone. In contrast, the sex steroids 17-beta estradiol and progesterone failed to enhance the adipose conversion process (Table III).

Although a long exposure time to cortisol was used in most experiments, a 3-d exposure to this hormone was sufficient to enhance both the number of differentiated cells visible under microscope and the GPDH activity (Table IV). Increasing the exposure time to cortisol up to 18 d led to a moderate increase in that activity. The single addition of MIX as a cAMP-elevating agent (21), during the first 3 or 6 d, was able to increase the GPDH activity in the absence or in the presence of cortisol. The effect of MIX appears to be additive to that of cortisol (Table IV) and to accelerate the adipose conversion process (not shown). The triacylglycerol content of cells maintained under the optimal conditions of Table IV was increased 3.3-fold (100 μg/dish vs. 30 μg/dish) as compared with cells maintained in ITT medium. Since the culture medium did not contain exogenous lipids, the accumulation of triacylglycerol droplets was exclusively the result of de novo synthesis, in agreement with the high capacity of human adipocyte precursors to incorporate glucose into cellular lipids (13).

The comparison of adipose conversion obtained in serum-free and serum-containing media, both supplemented with cortisol (0.1 μM) and insulin (0.5 μM), revealed a striking difference. In serum-containing medium, the percentage of differentiating cells was much lower as compared with that obtained in serum-free conditions. GPDH activity was only 3–4% of the value obtained in serum-free medium (28±6 vs. 893±165 mU/mg protein, $P < 0.001$); similar results were obtained whether or not MIX was present during the first 3 d (38±8 vs. 1,069±183 mU/mg protein, $P < 0.001$). The protein content was 3.4-fold higher in cultures kept in serum-containing medium as compared with serum-free medium (168±51 vs. 49±16 μg protein/dish), suggesting that the sustained growth, which was visible under microscope, was induced by serum mitogenic factors.

Table II. Effect of Time of Incubation in Serum-containing Medium on GPDH Activity

Time of incubation in serum-containing medium	GPDH activity
h	*mU/mg protein*
Primary cultures	
4	658±120
16–20	585±135
40–48	463±78
90	154±36
180	81±14
First subculture	55±19
Second subculture	ND

ND, not detectable.
Stromal-vascular cells from human adipose tissue were seeded at 30,000/cm² and maintained in serum-containing medium for the time periods indicated. Primary cultures were kept for another 18 d in ITT medium supplemented with 0.1 μM cortisol. Secondary cultures (first and second subcultures) were grown to confluence in serum-supplemented medium, trypsinized, plated in serum-supplemented medium for 20 h and then shifted and maintained for 18 d under serum-free conditions as above. GPDH activity was measured as described in Methods (means±SE of three separate experiments performed on duplicate dishes).

Table III. Effect of Other Steroid Hormones on the Expression of GPDH Activity in Stromal-Vascular Cells Derived from Human Adipose Tissue

Hormone	GPDH activity
	% of the maximum
—	27±2
Cortisol	83±5
Dexamethasone	100
Aldosterone	70±4
17 β-estradiol	25±8
Progesterone	20±5

16 h after inoculation in serum-containing medium, cells were cultured in ITT medium in the absence or presence of 0.1 μM of the various steroid hormones. Cultures were harvested after 18 d for the determination of GPDH activity. The values expressed in percentage of the maximum specific activity for medians ± ranges for two independent experiments. The specific activities of GPDH were 86 and 212 mU/mg without hormone supplementation and, respectively, 360 and 724 mU/mg in the presence of 0.1 μM dexamethasone.

Table IV. Effect of Exposure Time to Cortisol and/or MIX on the GPDH Activity in Stromal-Vascular Cells from Human Adipose Tissue

Additions	Exposure time	GPDH activity
	d	*mU/mg protein*
—	—	161±17
Cortisol	3	438±101
Cortisol	6	484±86
Cortisol	18	568±73
MIX	3	254±52
MIX	6	291±58
Cortisol* + MIX	3	779±116
Cortisol* + MIX	6	936±145

* Cortisol was present during the whole incubation period (18 d). 16 h after inoculation in serum-containing medium, cells were subsequently cultured in ITT medium supplemented or not with cortisol (0.1 μM) and/or MIX (0.25 mM) for the times indicated. 18 d later, cells were harvested for the determination of GPDH activity. Means±SE of three separate experiments performed on duplicate dishes.

The number of stromal-vascular cells capable to differentiate into adipose cells, i.e., to express GPDH, was dependent upon the age of the donor. GPDH activities were measured in cultures obtained from 27 adults ranging from 20 to 83 yr in age and maintained for 18 d in ITT medium supplemented with 0.1 μM cortisol. As shown in Fig. 5 by plotting the GPDH specific activity measured at day 18 as a function of the donor age, there was a significant inverse relationship ($r = -0.787$, $P < 0.001$). This inverse relationship was observed for both sexes (males, $r = -0.832$, $P < 0.001$; females: $r = -0.674$, $P < 0.01$) and led also to a reduction in the variability between subjects after 40–50 yr of age. The GPDH activities seemed to be slightly higher in cultures from male subjects than from female subjects, but a definite statement regarding this point cannot be made owing to disparities both in the number and the age distribution of male and female subjects.

Discussion

The present report defines the hormonal requirements for the differentiation of stromal-vascular cells, isolated from human adipose tissue, into adipose cells. These in vitro studies were based upon the chemically defined serum-free medium (ITT medium) recently developed by Deslex et al. (13, 22). In contrast to serum-containing media, up to 70% of the stromal-vascular cells obtained from young adults were found to convert into adipose cells in ITT medium when supplemented with glucocorticoids. This finding is in line with our previous studies performed in serum-containing medium which showed that glucocorticoid hormones, in the presence of insulin, play the role of adipogenic factors able to induce the adipose conversion process in human cells (12). The situation described herein for human cells is similar to the findings reported for stromal-vascular cells isolated from adipose tissue of young rats where most of the stromal-vascular cells, if not all, were able to convert into adipose cells (22, 23). The early development of LPL, as compared with that of GPDH and also with triacylglycerol accumulation, is in very good agreement with the situation already described in mouse preadipocyte cells (24–26). Thus LPL appears to be also an early marker of differentiation in human preadipocytes.

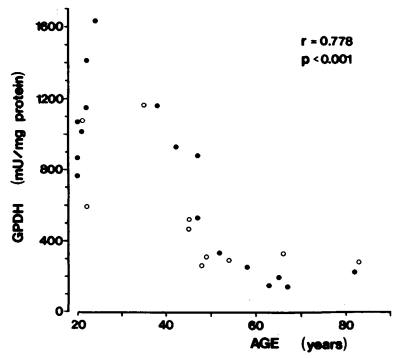

Figure 5. Correlation between age and GPDH activity expressed in cultured stromal-vascular cells from adipose tissue samples of 27 adult individuals. Stromal-vascular cells isolated from adipose tissue of 17 male (●) and 10 female (○) were cultured for 18 d in ITT medium supplemented with 0.1 μM cortisol before harvesting for measurement of enzyme activity.

In agreement with previous studies performed with rat and sheep adipose precursor cells in primary culture (22, 27, 28), our data indicate also that the presence of serum reduces dramatically the frequency of adipose conversion accompanied by a decrease of the GPDH activity by > 95% as compared with serum-free conditions. Among hypotheses to explain the lower frequencies of adipose conversion so far reported for rodent and human adipose precursor cells in serum-supplemented media (8–13, 26), it could be envisioned that the high mitogenic potency of serum, which is reflected by a significant increase in the cell number, leads by means of over-proliferation to a loss of specific early markers of preadipose cells, e.g., to a cell "decommitment." This phenomenon was reported to be the case in Ob17 preadipose cells (24, 29). Alternatively, it cannot be excluded that antiadipogenic factors are indeed present in serum: for instance tumor necrosis factor and transforming growth factor-β have been shown to decrease the expression of GPDH mRNA in Ob17 adipose cells independently of a growth-promoting activity (29), and this could also well be the case in other observations showing the inhibitory effects of the tumor necrosis factor, transforming growth factor-β, fibroblast growth factor and platelet-derived growth factor on adipose conversion (30–33).

It should be noted that stromal-vascular cells from human adipose tissue inoculated and maintained in the absence of serum, are able to differentiate to a similar extent as shown in this study where cells were inoculated in serum-containing medium for the attachment period and maintained in serum-free medium (Hauner, H., and G. Entenmann, manuscript in preparation). The high proportion of cells able to develop the adipocyte phenotype under our defined culture conditions indicates that the stromal-vascular fraction is much less heterogenous than originally thought (8–11) and that adipose tissue from donors at any age contains a remarkable pool of adipose precursor cells. The decrease in the capacity of differentiation as a function of age is likely related to the decreased susceptibility of the remaining precursor cells to respond to extracellular signals and/or a decreased proportion of these precursor cells. Such age-dependency of differentiation capacity has been also observed in rat (22, 34, 35) and at a given age with respect to the anatomical site of the fat depot (36). The state at which these adipose precursor cells are present in vivo remains un-

known. However, it must be recalled that dormant preadipose cells, containing pOb24 mRNA as an early marker, have been characterized in various adipose tissues of very old mice (25). Taken together, these observations suggest that dormant preadipose cells are present in rodent and human adipose tissues and are involved in the acquisition of new fat cells during adult life (3, 11, 37, 38).

The function of glucocorticoids for the development of adipocytes from precursor cells has been originally suggested by Rubin et al. (39) and recently investigated by Wiederer and Löffler (23). Glucocorticoid hormones, further identified as the main components of the adipogenic activity present in human serum (40), are indeed of great importance for the adipose conversion of stromal-vascular cells derived from human adipose tissue. The development of a fibroblast-like precursor cell to a mature fat cell is accompanied by a controlled sequence of changes in gene expression reflected by the de novo synthesis of enzymes involved in lipogenesis and other differentiation-specific proteins (41–43), in which regulation of the expression of various genes by glucocorticoids has been described (43, 44). The positive effect of glucocorticoids on adipose conversion is amplified by a cAMP-elevating agent (Table IV). In that respect, it is interesting to note that a sustained level of cAMP, coupled to the activation of the polyphosphoinositide breakdown, has been shown recently to be required for the differentiation of mouse Ob17 cells in serum-free medium (20). Furthermore, carbaprostacyclin, a stable analogue of prostacyclin known to be synthesized by murine and human preadipocytes, which is a potent activator of cAMP production and which has been shown to trigger terminal differentiation of Ob17 cells behaves also as an adipogenic factor for the differentiation of human stromal-vascular cells in ITT medium (45). Since glucocorticoids behave as mitogenic-adipogenic stimuli for Ob17 cells having expressed early markers (Gaillard, D., M. Wabitsch, and R. Négrel, manuscript in preparation), it is suggested that in man, like in mouse, glucocorticoids modulate the terminal differentiation of dormant preadipose cells.

The present culture system may provide a new tool to study, on the one hand the contribution of the human adipocyte precursor pool to the pathogenesis of obesity, and on the other hand, the hormonal control of the recruitment of new fat cells in man, including the possible involvement of paracrine/autocrine factors.

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