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Differentiation of Adipose Stromal Cells: The Roles of Glucocorticoids and 11β-Hydroxysteroid Dehydrogenase*

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ABSTRACT

Glucocorticoids play an important role in determining adipose tissue distribution and function, with glucocorticoid excess states such as Cushing's syndrome resulting in central obesity. We have investigated the functional significance of local generation of cortisol within adipose tissue from inactive cortisone through the activity of the NADP(H)-dependent enzyme, 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1).

In primary cultures of paired omental (om) and sc human adipose stromal cells (ASC; n = 34), 11βHSD1 oxo-reductase activity was significantly higher in om ASC (median, 40.2 pmol/mg protein·h; 95% confidence interval, 1.8–105) compared with sc ASC (median, 11.4; 95% confidence interval, 0–48.1; $P < 0.001$) despite similar endogenous NADPH/NADP concentrations. Both cortisol and insulin increased the differentiation of ASC to adipocytes (as assessed by glycerol-3-phosphate dehydrogenase expression), but only cortisol increased 11βHSD1 activity and messenger RNA levels in a dose-dependent fashion. Cortisone (500 nM) was as effective as 500 nM cortisol in inducing ASC differentiation, but this stimulatory effect was inhibited by coincubation with the 11βHSD1 inhibitor, glycyrrhetinic acid.

The higher local conversion of cortisone to active cortisol through expression of 11βHSD1 in om compared with sc ASC may explain the specific action of glucocorticoids on different adipose tissue depots. 11βHSD1 expression in om ASC is regulated at a transcriptional level and is increased by glucocorticoids, but is not entirely dependent upon ASC differentiation. Inhibition of 11βHSD1 within om ASC inhibits cortisone-induced ASC differentiation. These findings indicate that local metabolism of glucocorticoid may control differentiation of adipose tissue in a site-specific fashion. Specific inhibitors of 11βHSD1 may offer a novel approach for the treatment of patients with central obesity. (*Endocrinology* **140:** 3188–3196, 1999)

OBESITY occurs as a result of an expansion of adipose tissue mass by either hypertrophy of existing adipocytes or differentiation of preadipocytes [adipose stromal cells (ASC)] through to adipocytes. This prevalent condition is an established risk factor for premature mortality from cardiovascular disease, diabetes mellitus, hypertension, hyperlipidemia, and, at least in women, some malignancies (1). A series of epidemiological studies has suggested that for a given body mass index, mortality is higher if adipose tissue is deposited centrally (visceral or omental obesity) compared with generalized obesity (2). This has highlighted the importance of identifying factors determining adipose tissue distribution in addition to function and quantity. As exemplified in patients with Cushing's syndrome or in patients receiving corticosteroid therapy, glucocorticoids play an important role in regulating human adipose tissue distribution and function. Such patients develop an increase in visceral fat, often at the expense of sc fat (3, 4). *In vitro*, glucocorticoids are required for the differentiation of ASC to mature adipoctyes (5, 6) and also directly regulate the function of mature adipocytes (7, 8). The importance of glucocorticoids in obesity has been illustrated in some rodent models of obesity; adrenalectomy in the *ob/ob* mouse, for example, prevents the development of obesity (9).

A key factor in the analysis of corticosteroid hormone action is the activity of the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD). Two isozymes of 11βHSD catalyze the interconversion of hormonally active cortisol (F) and inactive cortisone (E) (10–12). 11βHSD type 1 (11βHSD1) is a low affinity NADP(H)-dependent enzyme that is predominantly expressed in human liver, adrenal, gonad, and decidua. By contrast, 11βHSD2 is a high affinity NAD-dependent enzyme that inactivates F to E in mineralocorticoid target tissues, such as kidney and colon (11, 12), thereby preventing illicit occupancy of the mineralocorticoid receptor (MR) by F (13, 14). 11βHSD1 expression has been shown to facilitate glucocorticoid hormone action in liver, skin, gonad, and central nervous system tissues (15, 16). We have recently described 11βHSD1 immunoreactivity in human adipose tissue (17) and have demonstrated that activity at this site is predominantly reductase (*i.e.* E to F ≫ F to E); reductase activity is higher in cultured omental (om) ASC compared with abdominal sc ASC (18). This suggests that om adipose tissue has a greater capacity to generate F from E compared with sc adipose tissue, and based on observations from patients with Cushing's syndrome, this may be an underlying factor in the pathogenesis of central obesity. The aim of this study was to further investigate the role of

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11βHSD1 expression in human om and sc fat depots. Specifically, we have evaluated the functional consequences of altered 11βHSD1 expression on adipose tissue differentiation.

Materials and Methods

Primary cell culture (ASC)

Human preadipocytes, ASC, were isolated from abdominal adipose tissue from sc and om depots. Paired samples from a total of 34 subjects (20 males; mean age, 53 yr; range, 24–78; mean weight, 71 kg; range 55–95) undergoing elective abdominal surgery (usually cholecystectomy, fibroids, or malignancy localized to the colon) were studied. The study had the full approval of the local hospital ethics committee. Dry tissue was collected and washed in 1 × HBSS with penicillin and streptomycin. Fat was chopped under sterile conditions into 2-mm pieces and digested with 2 mg/ml collagenase class I (Worthington Biochemical Corp., Freehold, NJ) in a shaking water bath at 37 C for 1 h. After dissociation, the fat tissue was pipetted through sterile cotton gauze to remove undigested connective tissue. The suspension was then centrifuged for 5 min at 1000 rpm (80 × *g*) at room temperature to separate preadipocytes (forming a cell pellet at the bottom of the tube) from adipocytes (in the supernatant). The supernatant was discarded and preadipocytes were resuspended in growth medium and plated in 6- or 12-well tissue culture plates (~1–2 g/plate). Cells were allowed to adhere to plastic overnight in DMEM-Ham's F-12 medium (DMEM/F12) with 15% FBS (Life Technologies, Paisley, UK). The next day, cells were washed and cultured until confluence (7–12 days) in growth medium (control) or growth medium supplemented with variable concentrations of insulin (1–500 ng/ml) and/or F (10 nM-1 μM). For differentiation experiments, cells were allowed to adhere overnight in DMEM/F12 with 15% FBS, washed, and grown until confluence in serum-free DMEM/F12 containing attachment supplement (insulin, transferrin, and selenium; Life Technologies) (5). For 7 days postconfluence, cells were cultured in serum-free medium with the addition of 500 nM F or E and with or without 5 μM glycyrrhetinic acid (GE), which was previously shown to totally inhibit 11βHSD1 activity (19).

11βHSD1 assays

Monolayers of ASC were washed in 1 × HBSS, and 1 ml fresh serum-free DMEM/F12 with 0.25 μM E and less than 1.5 nM [^{3}H]E tracer [synthesized in-house as previously reported (11, 19)] was added to each well. Cells were incubated in air-5% CO_2 at 37 C for 4 h, and the medium was transferred to a 10-ml glass tube. Cells were lysed, and protein concentrations were measured using a modification of the Bradford method (Bio-Rad Laboratories, Inc., München, Germany). These conditions ensured first order kinetics for all of the 11βHSD1 activity studies (11, 19).

Steroids were extracted from the medium in 10 vol dichloromethane and separated by TLC using chloroform-absolute ethanol (92:8) as the mobile phase. The TLC plates were scanned using Bioscan, Inc. 200 image detector (Lablogic, Sheffield, UK), and the fractional conversion of E to F was calculated and expressed as picomoles of F per mg total protein/h. Enzyme activity studies were carried out in triplicate for each individual patient preparation.

Glucose-6-phosphate dehydrogenase assay (G6PDH)

To investigate whether changes in the activity of the pentose phosphate pathway (dictating the intracellular NADPH/NADP ratio) could account for any differences in the relative expression of 11βHSD1 in om *vs.* sc ASC, G6PDH activity assays were carried out in parallel with 11βHSD1 activity studies (n = 4, in triplicate). G6PDH was measured using a commercially available kit (Biozyme Laboratories Ltd., Blaenavon, UK). Briefly, cultured cells were lysed, scraped in 100 μl triethanolamine buffer (TEA; 0.1 M; pH 8), and transferred to Eppendorf tubes. After a protein assay, G6PDH activity was analyzed in a 1-ml total volume of TEA buffer with 20 μg protein, glucose-6-phosphate (1.27 mM), $NADP^+$ (0.44 mM), and $MgCl_2$ (6.6 mM). Optical density changes at 340 nm were measured for 30 min at 5-min intervals and expressed as G6PDH units per mg protein/min; 1 U is the amount of enzyme that caused the reduction of 1 μmol $NADP^+$/min at 25 C and pH 8.

RNA extraction

After enzyme assays, total RNA was extracted from ASC using a single step extraction method (RNAzol B, AMS Biotechnology, Witney, UK). The glucocorticoid receptor (GR) and MR expression studies were undertaken on freshly isolated ASC or adipocytes from om and sc sites as described above. Positive controls employed stored RNA samples from human liver and colon. Unless otherwise stated, all other reagents for the RNA analyses were purchased from Promega Corp. (Southampton, UK). RNA integrity was confirmed by electrophoresis on 1% agarose gels, and concentration was assessed by spectrophotometry at OD_{260}.

RT reaction

Total RNA (0.5 μg) was denatured by heating to 70 C for 10 min. Fifteen units of avian myeloblastosis virus, 100 ng random hexamers, 10 U ribonuclease inhibitor, and 20 nmol deoxy-NTPs with 5 × reaction buffer were added to a total volume of 20 μl. The reaction was performed at 37 C for 1 h, and complementary DNA (cDNA) was heated at 95 C for 10 min to inactivate any remaining enzymes. For synthesizing GR and MR cDNA template, the above RT protocol was modified, and 0.5 μg oligo(deoxythymidine)$_{15}$ primers were used at 42 C for 1 h.

Relative RT-PCR of 11βHSD type 1, MR, and GR messenger RNA (mRNA)

QuantumRNA (Quantitative RT-PCR Module, Ambion, Inc., Austin, TX), was used to analyze 11βHSD1 mRNA levels in ASC between individual treatments (n = 4). Aliquots (0.5 μg) of total RNA were reverse transcribed using random hexamers as described above. 18S ribosomal rRNA (18S rRNA) and 11βHSD type 1 DNA fragments of 488 and 571 bp, respectively, were amplified in a one-tube multiprimer PCR reaction (denaturation at 94 C, annealing at 50 C, and extension at 72 C, 1 min each for 35 cycles), using human 11βHSD1 primers as previously described (20). Similarly, using previously reported methods (20), RT-PCR analysis of GR mRNA expression was carried out using the following primers: sense, 5′-TCGACCAGTGTTCCAGAGAAC-3′; and antisense, 5′-TTTCGGAACCAACGGGAATTG-3′. Amplification of a 693-bp fragment was performed using an initial denaturation cycle of 95 C (5 min) followed by 30 cycles of 94 C (1 min), 55 C (1 min), and 72 C (1 min). Analysis of MR mRNA expression was carried out using the following primers: sense, 5′-AACTTGCCTCTTGAGGACCAA-3′; and antisense, 5′-AGAATTCCAGCAGGTCGCTC-3′. Amplification of a 471-bp fragment was performed using an initial denaturation cycle of 95 C (5 min), followed by 30 cycles of 94 C (1 min), 54 C (1 min), and 72 C (2 min). A final elongation step of 72 C for 5 min was included for both GR and MR PCR amplifications. To test the efficiency of the RT reaction, a 468-bp fragment of β-actin, a housekeeping gene, was amplified using 10 pmol of each sense primer (5′-GTCACCAACTGGGACGACA-3′) and antisense primer (5′-TGGCCATCTCTTGCTCGAA-3′) in 10 × reaction buffer with 0.25 mM $MgCl_2$. Amplification was carried out for 30 cycles with a denaturing temperature of 94 C for 1 min, an annealing temperature of 60 C for 1 min, and an extension step at 72 C for 2 min.

18S rRNA primers were used at a 3:7 ratio to 18S PCR competimers, which ensured linear amplification of this cDNA. Multiprimer amplification of target and endogenous internal control cDNAs, both of similar size, enabled a direct comparison between multiple samples by measuring the relative abundance of target transcript when separated on a 2% MetaPhor agarose gel (FMC Bioproducts, Rockland, ME). The image of the UV-illuminated gels was stored in digital form and analyzed using UVGel software (UV GelBase, UVP Ltd., Cambridge, UK).

G3PDH quantitative (competitive) RT-PCR

Levels of G3PDH mRNA were used as a marker of adipocyte differentiation as previously reported (21). A quantitative (competitive) RT-PCR approach employed an exogenous mutant G3PDH comple-

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mentary RNA as an internal control (n = 4). This mutant G3PDH complementary RNA differed from wild-type G3PDH by having a deleted *Eco*RI restriction site, permitting the resolution of wild-type (198-bp) and mutant (220-bp) PCR products when digested with 1 U *Eco*RI/μl PCR product on 3% (FMC, Rockland, ME) agarose gel. After a series of studies to define the linearity of this reaction, 5 pg mutant G3PDH/0.5 μg total RNA were transcribed using random hexamers as described above. Two microliters of this 50-μl reaction were used for amplification (denaturation at 94 C, annealing at 60 C, and extension at 72 C for 1 min each, 35 cycles). The relative changes in wild-type/mutant mRNA levels were assessed by measuring the area ratios in amplified DNA fragments using an image analyzer and UVGel software.

G3PDH enzyme assay

The G3PDH enzyme assay was carried out using a previously reported methodology (22). Monolayers of cultured human ASC were disrupted by storage at −80 C. Enzyme assays were performed in a 1-ml total volume of 0.1 M TEA buffer (pH 7.6) with 0.1% BSA, 0.8 mM dihydroxyacetone phosphate (substrate), 0.2 mM NADH, and 10 μg cellular protein. The rate of decrease in absorbance at 340 nm was followed spectrophotometrically for 30 min at 5-min intervals. In each assay, dilutions of a G3PDH standard (0.0125 U/ml; Sigma Chemical Co.) were analyzed, and G3PDH activity was expressed as units per mg protein; 1 U is the amount of enzyme that catalyzed the conversion of 1 nmol dihydroxyacteone phosphate to α-glycerophosphate/min at 25 C (pH 7.6).

Data were expressed as the median and 5th-95th percentile (for nonparametric data) or as the mean ± SE (for normally distributed data), and statistical analysis between groups was performed as appropriate, using either the Mann-Whitney U test or Student's *t* test.

Results

In 34 paired om and sc adipose stromal cell preparations, 11-oxo-reductase activity was significantly higher in om compared with sc sites [median, 40.2 (95% confidence interval, 1.8–104) pmol/mg·h *vs.* 11.4 (0–48.1); $P < 0.001$]. Coincubation with 100 nM F increased oxo-reductase activity at both sites, but again the stimulated activity was highest in om ASC [median, 84.2 (95% confidence interval, 18.4–257) *vs.* 31.6 (0.0–126); $P < 0.001$; Fig. 1]. Dose-response studies carried out on om ASC cells indicated that this induction of 11-oxo-reductase activity was maximal at F concentrations of 500-1000 nM (Table 1).

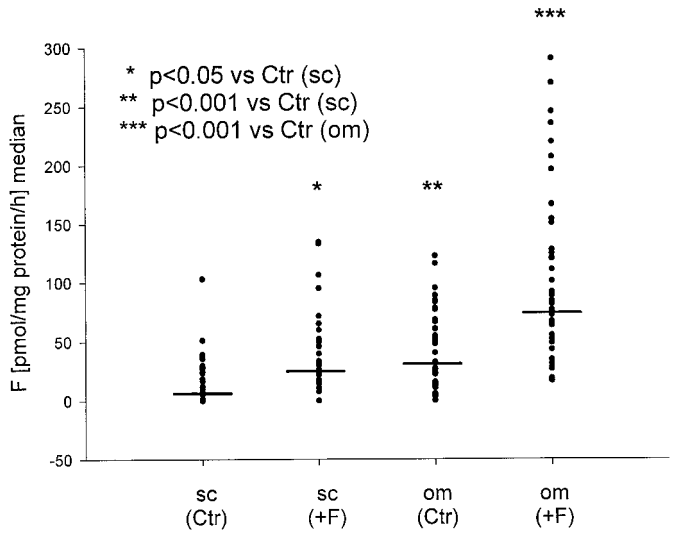

FIG. 1. 11-Oxo-reductase activity, represented as picomoles of F per mg protein/h, in primary cultures of human adipose stromal cells obtained from om or sc depots (n = 34). Median values for each group are given. For each primary culture, cells were incubated either with (+F) or without (Ctr) F (100 nM).

TABLE 1. Effect of cortisol on 11βHSD1 oxo-reductase activity in primary cultures of human om ASC

Treatment	11βHSD1	n
Control	100.0	8
10^{-8} M F	100.6 ± 2.9	3
10^{-7} M F	230.9 ± 37.8[a]	8
2.5×10^{-7} M F	318.1 ± 29.8[b]	3
5×10^{-7} M F	392.1 ± 66.9[b]	3
10^{-6} M F	470.7 ± 41.3[a]	8

Results are expressed as the mean ± SE percentage of the control value (=100%). The number of experiments (n) is shown; each experiment was carried out in triplicate. Control conversion was 65.2 ± 10.1 pmol/mg protein · h.

[a] $P < 0.001$ *vs.* control.

[b] $P < 0.05$ *vs.* control.

Endogenous NADPH/NADP ratios within ASC from om and sc fat depots were similar, as reflected in the G6PDH activity studies. Thus, in sc and om ASC, G6PDH activities were 6.1 ± 0.8 and 5.3 ± 0.9 U/mg protein·min, respectively (n = 4 in triplicate), and were not altered by coincubation with 100 nM F (5.7 ± 0.4 *vs.* 4.7 ± 0.7; Fig. 2, a and b).

RT-PCR analysis indicated expression of GR mRNA, but not MR, mRNA in both om and sc adipocytes (Fig. 3). After 35 cycles of PCR, very weak expression of MR mRNA was observed in sc and om ASC. The relative expression of GR mRNA was much higher (Fig. 3).

The F-mediated induction of 11βHSD1 activity (control, 38.5 ± 8.6; F, 110 ± 25.5 pmol/mg·h; Fig. 4a) and 11βHSD1 mRNA (control, 22.2 ± 3.2; F, 45.1 ± 9.2 11βHSD/18S, arbitrary units; Fig. 4, b and c) was not entirely dependent upon the process of adipocyte differentiation. Using G3PDH as a marker of ASC differentiation, cotreatment with insulin and F resulted in an independent and synergistic increase in ASC differentiation (control, 1.0 ± 0.9; insulin, 11.2 ± 0.7; F, 43.4 ± 3.1; F plus insulin, 79.3 ± 11.3, wild-type/mutant G3PDH mRNA; Fig. 5), which was also reflected in G3PDH enzyme activity (control, 3.9 ± 0.7; insulin, 5.5 ± 1.0; F, 6.1 ± 0.6; F plus insulin, 7.0 ± 0.02 U/mg protein·min). Only F increased 11βHSD1 activity (Fig. 4a) and mRNA levels (Fig. 4b); insulin had no effect (*e.g.* 11βHSD1 activity with F was 110 ± 25.5 and with F plus insulin was 97.3 ± 20.4 pmol/mg·h; Fig. 4a).

The functional importance of 11βHSD1 in regulating this glucocorticoid-mediated increase in ASC differentiation is demonstrated in Fig. 6. F and E in doses of 500 nM caused a similar induction of G3PDH activity (control, 3.5 ± 0.5; F, 7.8 ± 0.9; E, 6.1 ± 0.5 U/mg protein·min). GE (5 μM) itself had no effect on ASC differentiation (control plus GE, 4.1 ± 0.6 U/mg protein·min), but by blocking the conversion of E to F resulted in an inhibition of E-induced ASC differentiation (3.9 ± 0.7 U/mg protein·min; Fig. 6, a and b). GE did not inhibit F-induced ASC differentiation (data not shown).

Discussion

The GR is expressed in adipose tissue (23–25), and glucocorticoids are known to be required for the differenti-

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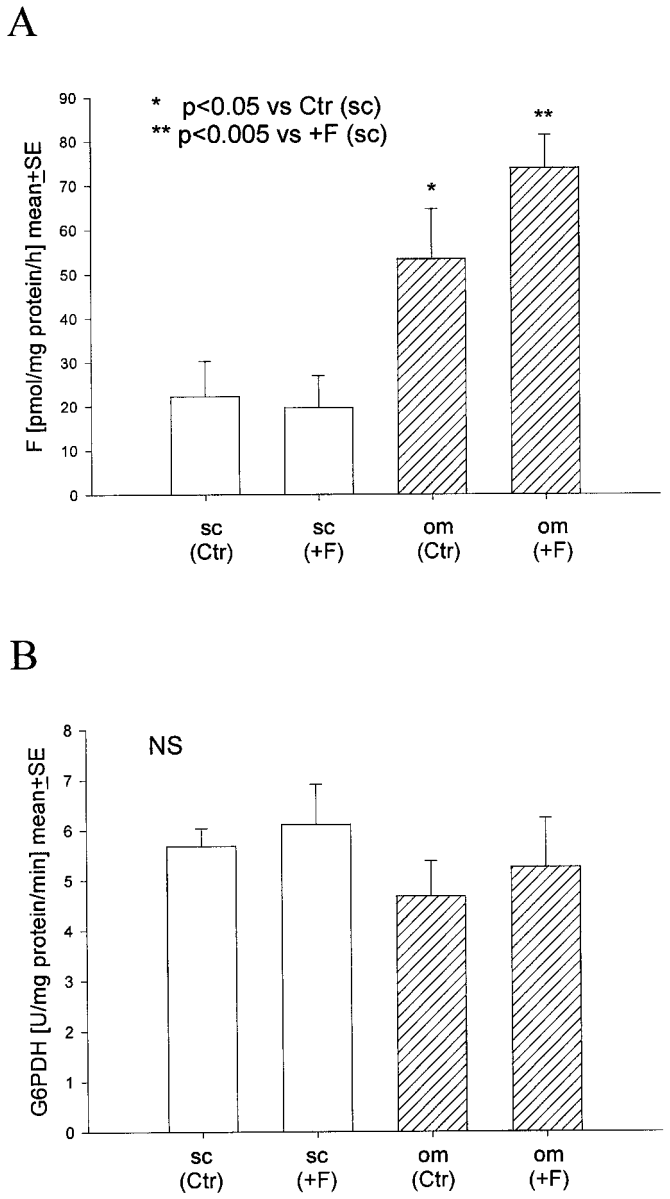

FIG. 2. Parallel analyses of 11βHSD1 oxo-reductase activity (A) and G6PDH activity (B) in primary cultures of human om and sc adipose stromal cells (n = 4, in triplicate). Results are depicted as the mean ± SE. 11βHSD1 activity is significantly higher in om ASC, but G6PDH values are similar at both sites.

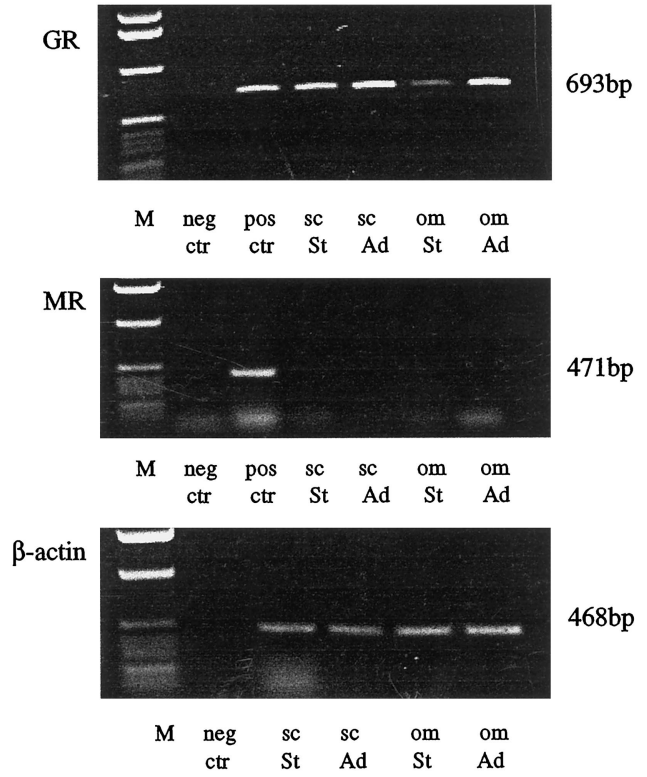

FIG. 3. RT-PCR analysis of GR and MR mRNA in isolated adipose stromal cells (St) and adipocytes (Ad) from om and sc human adipose tissue. In each RT-PCR reaction, negative controls comprised the absence of cDNA template. The positive control for 11βHSD1 was RNA from human liver, and that for 11βHSD2 was RNA from human colon. Compared with β-actin expression, GR mRNA was found in both om and sc ASC and adipocytes. No mRNA for MR was observed in adipocytes, and very low expression was seen in ASC (35 cycles).

ation of ASC into mature adipocytes (5, 26). Thus, glucocorticoids increase lipid accumulation in primary cultures of human ASC and the murine 3T3-L1 preadipocyte cell line through a process involving the induction of enzymes, including lipoprotein lipase (27, 28) and G3PDH (26). Once differentiation has occurred, glucocorticoids are also known to regulate the expression of several novel adipocyte gene products, including components of the local renin-angiotensin system, leptin (7), and peroxisome proliferator-activated receptor (8). Clinically, this profound effect of glucocorticoids on adipocyte differentiation and function is exemplified in the obesity observed in patients with Cushing's syndrome or in those taking corticosteroids. A crucial observation in such patients, however, is the predilection for central or visceral obesity (3, 4). This differential effect of glucocorticoids on adipose tissue depots has not been fully explained, although levels of mRNA for the GR have been reported to be higher in omental adipose tissue (24). The elucidation of the underlying mechanisms controlling this process is likely to have major ramifications for patients with obesity, particularly patients with central obesity or the so-called metabolic syndrome, who, for a given body mass index, have increased premature mortality compared with patients with generalized obesity (2). Our study suggests that this discrepant effect of glucocorticoids on body fat distribution may be regulated through the metabolism of glucocorticoids within adipose tissue itself. Thus, in paired sc and om ASC preparations from 34 subjects, generation of the active glucocorticoid, F, from inactive E via 11βHSD1 activity was significantly higher in om ASC compared with sc ASC, confirming our earlier preliminary observations (18). *In vivo* this would result in higher local concentrations of F within om ASC compared with sc ASC. Our studies suggest that F generated locally in this way may increase the differentiation of ASC to mature adipocytes. There was a marked interindividual variability in both the expression and glucocorticoid induction of 11βHSD1 in omental ASC,

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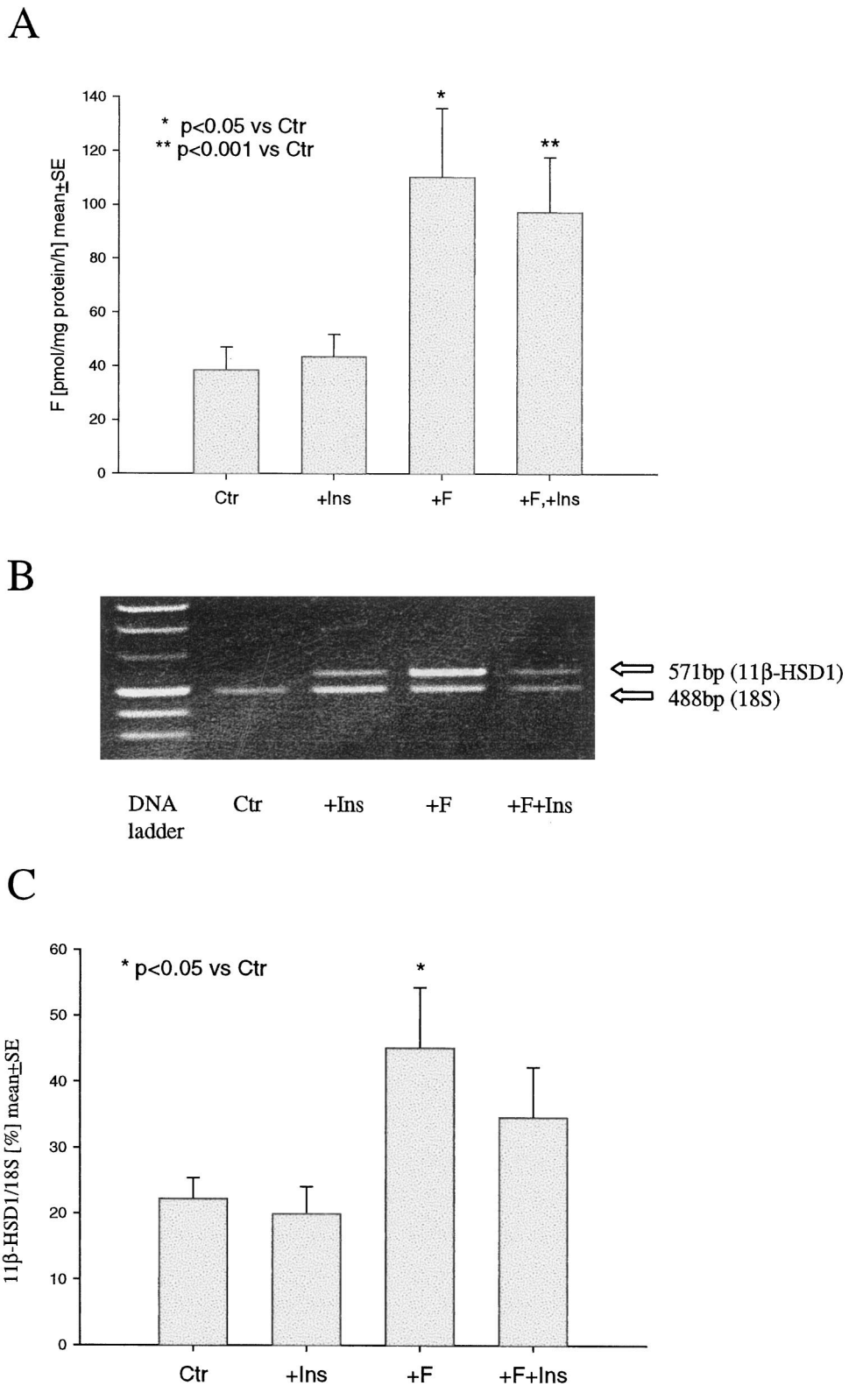

FIG. 4. The effects of F (100 nM) and insulin (50 nM) on 11βHSD1 oxo-reductase activity and mRNA levels in primary cultures of human om adipose stromal cells (n = 4). Activity results are depicted in A (mean ± SE), and results of RT-PCR 11βHSD1 mRNA studies are shown in B. C demonstrates the densitometric quantification of the data depicted in B. F significantly increases 11βHSD1 oxo-reductase activity and 11βHSD1 mRNA levels (B and C), but insulin has no effect.

and it is exciting to speculate that this may be a novel susceptibility factor, explaining the predisposition of some individuals, but not others, to central obesity.

There are many precedents for the autocrine or paracrine control of hormone action through the local metabolism of steroid hormones, for example 5α-reductase and the actions of testosterone (29) and aromatase and the peripheral activity of estrogens (30). Perhaps the best example, however, relates

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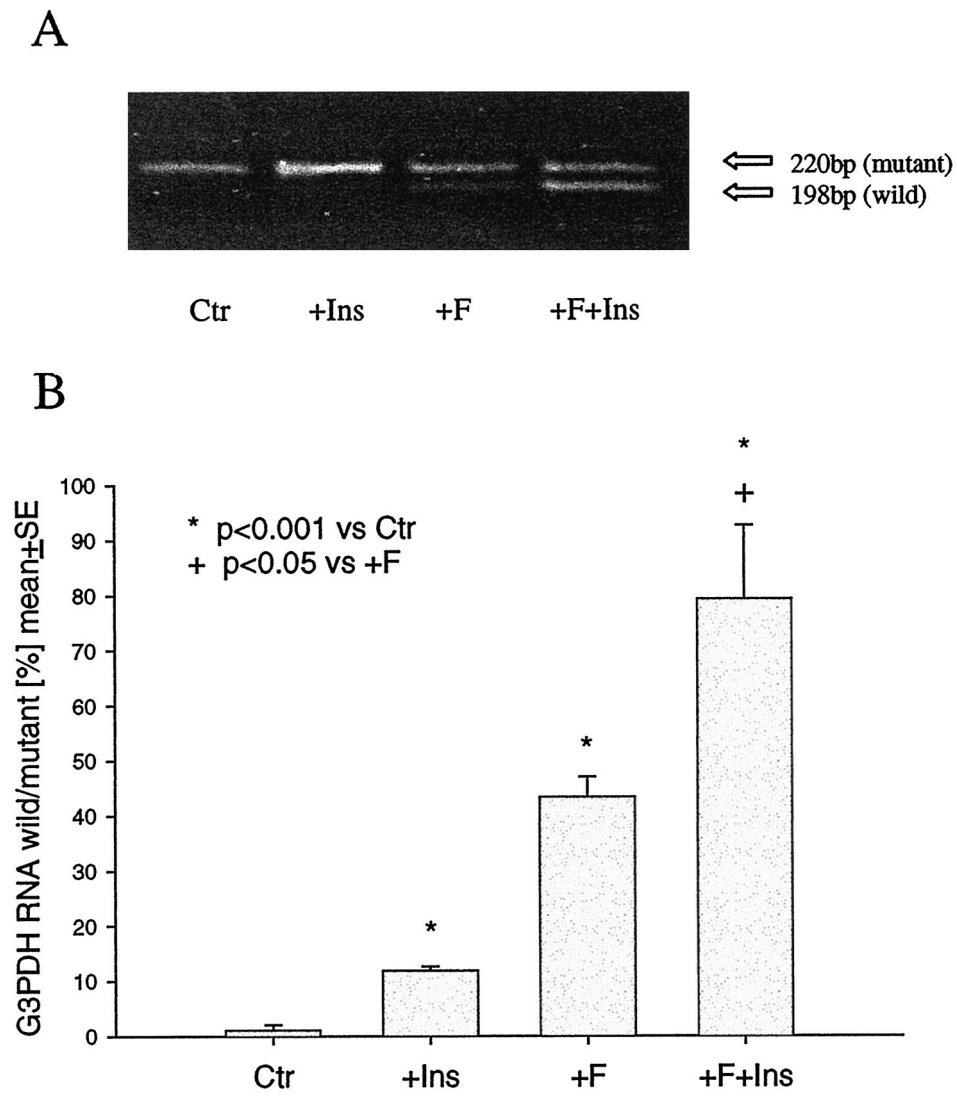

FIG. 5. The effects of F (100 nM) and insulin (50 nM) on G3PDH mRNA (a marker of adipocyte differentiation) in primary cultures of human omental adipose stromal cells. Experiments were carried out in an identical fashion as those reported in Fig. 4. Quantitative RT-PCR analysis of G3PDH mRNA indicated a synergistic stimulation of adipocyte differentiation with insulin and F.

to 11βHSD and corticosteroid hormone action. 11βHSD2 is a high affinity dehydrogenase expressed in kidney, colon, and salivary gland. This enzyme inactivates F to E, enabling aldosterone to bind to the MR *in vivo* (13, 14). Deficiency of 11βHSD2, either inherited in the syndrome of apparent mineralocorticoid excess (31, 32) or acquired after licorice ingestion (33), results in F gaining access to the MR to act as a potent mineralocorticoid. By contrast, 11βHSD1 acts as a reductase *in vivo*, generating F from E, and has been shown to facilitate glucocorticoid hormone action at keys sites, including the liver and central nervous system (15, 16). Circulating E is principally derived from the activity of renal 11βHSD2, with circulating concentrations approximately 1/10th those of F (34). However, E has a much lower affinity for F-binding globulin than F, and free levels are probably more similar. The characterization of 11βHSD within adipose tissue has shown the presence of 11βHSD1, but not 11βHSD2 (17, 18), in keeping with a functional role for 11βHSD1 in modulating tissue glucocorticoid hormone action. Specifically, our data suggest a crucial role for 11βHSD1 in regulating the process of glucocorticoid-induced ASC differentiation. E, through its conversion to F by 11βHSD1, was shown to stimulate ASC differentiation as effectively as F itself. Inhibition of 11βHSD1 by GE, the active component of licorice, resulted in a significant inhibition of E-induced ASC differentiation. GE is known to inhibit 11βHSD2 as well as 11βHSD1 (11, 12, 35) and was used in these experiments in the absence of a specific 11βHSD1 inhibitor. Although aldosterone has been shown to induce differentiation of the mouse 3T3-L1 cell line via the MR (36), we were unable to demonstrate appreciable levels of MR mRNA in either ASC or adipocytes. By contrast, abundant GR mRNA was expressed in keeping with earlier observations (23, 25), and it seems likely, therefore, that 11βHSD1 is indeed modulating active glucocorticoid exposure to the GR.

11βHSD1 is a NADP(H)-dependent enzyme, and it is of some interest that the principal sites of expression of this enzyme in human tissues are those with the highest redox potential (10, 17), manifested as the NADPH/NADP ratio. To investigate whether the availability of cofactor could be a rate-limiting factor in explaining the higher om compared with sc expression, assays of G6PDH, the first enzyme in-

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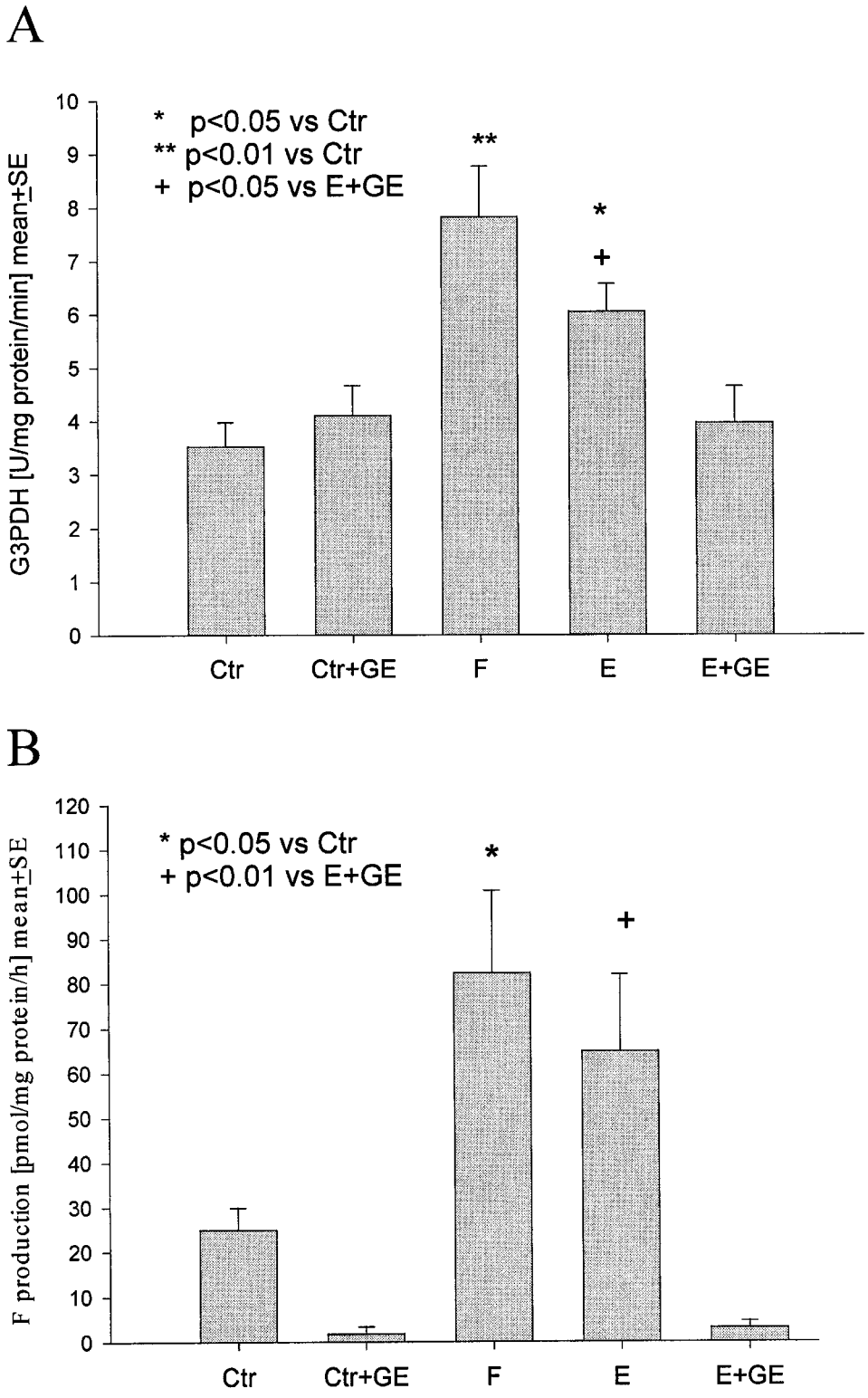

FIG. 6. The pivotal effect of 11βHSD1 on the differentiation of primary cultures of human omental adipose stromal cells to adipocytes (n = 5). In the presence of 500 nM F or E, adipocyte differentiation (as assessed by G3PDH activity; A) was significantly increased. GE at 5 μM inhibited 11βHSD1 activity (B), but had no effect on G3PDH activity. When E was coincubated with GE, no increase in G3PDH activity was observed.

volved in the pentose phosphate pathway, were undertaken. This pathway is responsible for the production of pentose phosphates for DNA and RNA biosynthesis and for the intracellular generation of reducing agent (NADPH) for pathways that require NADPH as an essential cofactor, such as 11βHSD1 and fatty acid synthesis. No differences were observed in G6PDH activity between om and sc sites, suggesting that endogenous cofactor concentrations are not rate limiting in determining 11βHSD1 expression. The control of 11βHSD1 mRNA and activity within ASC, however, was subject to "fast forward" feedback in a dose-dependent fashion by the substrate (F) itself, and this was more pronounced

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in om compared with sc ASC. Glucocorticoids have been shown to regulate 11βHSD1 expression at other sites (37, 38), and there are several putative glucocorticoid response elements within the promoter region of the human 11βHSD1 gene. Other factors known to regulate ASC function, such as CAAT enhancer-binding protein-α (C/EBPα), are also known to stimulate 11βHSD1 gene expression (39), and further studies addressing the transcriptional control of 11βHSD1 expression within human ASC are required. Insulin, however, failed to stimulate 11βHSD1 expression; on the contrary, it attenuated the glucocorticoid induction of 11βHSD1 mRNA and activity in keeping with earlier activity studies carried out on skin fibroblasts (40) and cultured hepatocytes (37, 38). This occurred despite stimulation of ASC differentiation in the presence of insulin and glucocorticoids, suggesting that the induction of 11βHSD1 expression in ASC upon exposure to F is not entirely dependent upon the differentiation process. It remains to be seen whether this inhibitory effect of insulin is of relevance in subjects with common insulin-resistant states such as type 2 diabetes mellitus.

In summary, the enhanced expression of 11βHSD1 and hence the generation of active F from E in om compared with sc ASC offers a novel explanation for the differential effects of glucocorticoids on regional depots of adipose tissue. The increased expression of 11βHSD1 in om ASC cannot be explained on the basis of an alteration in redox potential at this site. Its expression is increased by glucocorticoids and attenuated by insulin through a process that appears to be transcriptionally regulated and not entirely dependent upon differentiation of ASC to mature adipocytes. Inhibition of 11βHSD1 activity inhibits the differentiation of ASC by E; the future development of specific 11βHSD inhibitors may represent a novel approach in the treatment of metabolic disorders linked to central obesity. To date, there are no specific inhibitors of 11βHSD1 to test this hypothesis in clinical studies, but as the human 11βHSD1 and 11βHSD2 enzymes share only 14% identity (10, 12), it seems likely that selective inhibitors of 11βHSD1 will emerge.

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