Increased Glucocorticoid Receptor Expression in Human Skeletal Muscle Cells May Contribute to the Pathogenesis of the Metabolic Syndrome

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Altered glucocorticoid hormone action may contribute to the etiology of the metabolic syndrome, but the molecular mechanisms are poorly defined. Tissue sensitivity to glucocorticoid is regulated by expression of the glucocorticoid receptor (GR)-α and 11β-hydroxysteroid dehydrogenase type I (11β-HSD1)-mediated intracellular synthesis of active cortisol from inactive cortisone. We have analyzed GRα and 11β-HSD1 expression in skeletal myoblasts from men ($n = 14$) with contrasting levels of insulin sensitivity (euglycemic clamp measurements of insulin-dependent glucose disposal rate), blood pressure, and adiposity. Positive associations were evident between myoblast expression of GRα under basal conditions and levels of insulin resistance ($r^2 = 0.34$, $P < 0.05$), BMI ($r^2 = 0.49$, $P < 0.01$), percent body fat ($r^2 = 0.34$, $P < 0.02$), and blood pressure ($r^2 = 0.86$, $P < 0.001$). Similar associations were evident when myoblasts were incubated with physiological levels of cortisol ($P < 0.01$ for all). Importantly, GRα expression was unaffected by variations in in vivo concentrations of insulin, IGF-1, or glucose concentrations. In common with the GR, 11β-HSD1 expression in myoblasts incubated with physiological concentrations of cortisol in vitro was positively associated with levels of insulin resistance ($r^2 = 0.68$, $P < 0.001$), BMI ($r^2 = 0.63$, $P < 0.005$), and blood pressure ($r^2 = 0.27$, $P < 0.05$). Regulation of GRα and 11β-HSD1 by cortisol was abolished by the GR antagonist RU38486. In summary, our data suggest that raised skeletal muscle cell expression of GRα and 11β-HSD1–mediated regulation of intracellular cortisol may play a fundamental role in mechanisms contributing to the pathogenesis of the metabolic syndrome. *Diabetes* 51:1066–1075, 2002

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11β-HSD, 11β-hydroxysteroid dehydrogenase; DMEM, Dulbecco's Modified Eagle's Medium; GR, glucocorticoid receptor; hGR, human GR; HPLC, high-performance liquid chromatography; MR, mineralocorticoid receptor; SSC, sodium chloride–sodium citrate.

Metabolic resistance to insulin constitutes a major defect in the pathogenesis of type 2 diabetes and related conditions, including glucose intolerance, hypertension, endothelial dysfunction, and dyslipidemia (1,2). The combination of these abnormalities in the same patient is referred to as the metabolic, or insulin resistance, syndrome (3). Obesity is often strongly associated with features of the metabolic syndrome (4–6), which constitutes a significant risk factor for premature atherosclerosis and cardiovascular disease. Although obesity is known to cause insulin resistance, the pathogenesis of the metabolic syndrome remains poorly understood.

Skeletal muscle is a major target tissue for insulin-mediated glucose uptake, metabolism, and utilization in humans. Indeed, impaired insulin action in skeletal muscle is responsible for the majority of the decreased levels of nonoxidative glucose disposal that are observed in subjects presenting with the metabolic syndrome, obesity, and type 2 diabetes (7,8). Importantly, quantitative differences in insulin-stimulated glucose uptake and glycogen synthase activity in cultured skeletal myoblasts closely agree with those for in vivo glucose disposal and muscle glycogen synthase activity in type 2 diabetic and normal subjects from whom the myoblasts originated (9,10). This suggests that cultured skeletal myoblasts are a useful model system with which to investigate mechanisms underlying the pathogenesis of insulin resistance in humans.

Glucocorticoids are potent antagonists of insulin action and, when in excess, can promote insulin resistance and obesity (11). The metabolic and Cushing's syndromes share many features, suggesting that abnormalities of glucocorticoid hormone action may contribute to the pathogenesis of the metabolic syndrome (12). Increased glucocorticoid hormone action in skeletal muscle has been shown to impair insulin signaling through a variety of pathways (13,14), to an extent that mimics that observed in the profoundly insulin-resistant state. Glucocorticoids also induce gluconeogenesis (12,14,15), stimulate differentiation of functional fat cells from preadipocyte cells, and promote lipolysis and triglyceride storage, predominantly in the increased depots of visceral fat that are associated with the metabolic and Cushing's syndromes (16). This has led to the hypothesis that increases in glucocorticoid

hormone action may represent a common primary etiology underpinning these cardiovascular disease risk factors.

Conflicting data from case-control and cross-sectional analyses of circulating cortisol levels and hypothalamopituitary adrenal axis activities in men and women with contrasting levels of blood pressure, glucose intolerance, insulin resistance, and hyperlipidemia (15–20) have led to suggestions that the principal mechanisms underlying such increases in glucocorticoid hormone action are those regulating tissue sensitivity to circulating glucocorticoid. This is supported by studies describing the absence of Cushingoid features and metabolic abnormalities in hypercortisolemic patients (21) and, conversely, the manifestation of the Cushing's syndrome phenotype in hypocortisolemic patients (22).

The magnitude of tissue sensitivity to circulating glucocorticoid is regulated by levels of cortisol binding that are in turn largely determined by the expression of both the glucocorticoid receptor (GR) (23) and 11β-hydroxysteroid dehydrogenase (11β-HSD) (24). Recent studies indicate the ubiquitous expression of both the ligand-binding GRα and non–ligand-binding GRβ isoforms, which comprise splice variants from the same GR gene (23,25). Similarly, two isoforms of 11β-HSD have been characterized and cloned. 11β-HSD1, expressed predominantly in classical glucocorticoid target tissues, encodes low-affinity NADP(H)-dependent 11-oxoreductase activity, generating active cortisol from cortisone. In contrast, 11β-HSD2 encodes a NAD-dependent high-affinity unidirectional 11-dehydrogenase metabolizing cortisol to cortisone. 11β-HSD1 is thought to modulate glucocorticoid hormone action by regulating ligand supply to the GR (24). Recent human studies point to key roles for both the GR and 11β-HSD1 in the etiology of raised blood pressure, insulin resistance, hyperglycemia (12,26), and central obesity (27). Indeed, the insulin sensitivity of human volunteers has been shown to be significantly improved by pharmacological inhibition of 11β-HSD1 11-oxoreductase activity (28). The physiological importance of both the GR and 11β-HSD1 is further supported by animal studies in which pharmacological blockade of the GR abolishes high-fat diet–induced adiposity, glucose intolerance, and insulin resistance (29), and deletion of the 11β-HSD1 gene attenuates gluconeogenesis despite high circulating glucocorticoid levels (30).

We describe for the first time strong associations between in vivo glucose disposal, obesity, and raised blood pressure and the expression of GR and 11β-HSD in human skeletal muscle cells in vitro. These data suggest important molecular mechanisms underlying the pathogenesis of these key features of the metabolic syndrome.

RESEARCH DESIGN AND METHODS

A total of 14 adult men with BMI between 25 and 40 kg/m^2 were recruited for the study. Two subjects had type 2 diabetes, but none had evidence of other disease, and none were receiving medication. The experimental protocol was approved by the combined ethical committee of Southampton and Southwest Hampshire National Health Service Trust, and written informed consent was obtained from each subject. All of the nondiabetic subjects had normal glucose tolerance, defined by a fasting glucose <126 mg/dl and a 2-h glucose level after a standard 75-g oral glucose tolerance test of <140 mg/dl. Each subject was admitted to the Clinical Research Facility at Southampton General Hospital, where they consumed a standard weight-maintenance diet that comprised 55% of calories as carbohydrate, 30% as fat, and 15% as protein for at least 24 h before the studies. During this time, each subject completed a lifestyle and health questionnaire and underwent measurements of blood pressure, weight, height, waist, and hip circumference and skin-fold thickness by the same trained observer to enable calculation of BMI, waist-to-hip ratio, and percent body fat. These data, with the exception of glucose disposal rate, were found to be normally distributed and are summarized in Table 1 as previously described (31).

TABLE 1
Characteristics of the subject group

Characteristic	Arithmetic mean ± SD	Range
Insulin sensitivity (GDR) [(mg · m^{-2} · min^{-1}) · (μU · ml^{-1})]	5.7 ± 2.9	2.32–10.10
BMI (kg/m^2)	29.8 ± 4.3	25.2–39.3
Waist-to-hip ratio	0.967 ± 0.072	0.862–1.087
Percent body fat	26.3 ± 4.2	19.0–32.4
Systolic blood pressure (mmHg)	147.6 ± 28.9	110–206
Diastolic blood pressure (mmHg)	86.9 ± 11.3	74–110
Age (years)	58.6 ± 9.0	40–69

GDR, glucose disposal rate.

Hyperinsulinemic-euglycemic clamp. Insulin resistance indexes were measured using the hyperinsulinemic-euglycemic glucose clamp technique. All subjects fasted for 12 h overnight before the procedure. Insulin and glucose infusions were administered into an antecubital vein. Blood sampling was performed from a dorsal vein on the opposite hand. This hand was warmed to enable sampling of arterialized blood. After a priming infusion of insulin, a continuous infusion of insulin was started at a rate of 60 mU · m^{-2} · min^{-1}. The infusion was continued for 2 h. The plasma glucose was maintained at 5 mmol/l by variable glucose infusion. The amount of glucose to maintain euglycemia was taken as the amount of glucose metabolized (M). The mean plasma insulin (I) during steady-state euglycemia (60–120 min) was calculated. The M/I ratio [(mg · m^{-2} · min^{-1}) · (μU · ml^{-1})] was used as the measure of tissue sensitivity to insulin.

Skeletal muscle biopsy and cell culture. All reagents were obtained from Sigma-Aldrich (Poole, U.K.) and Gibco-Life Technologies (Paisley, U.K.), unless otherwise stated.

Tissue was obtained by Bergstrom needle biopsy (26–143 mg) of the vastus lateralis muscle in the thigh of 14 Caucasian men, the characteristics of whom are shown in Table 1. Two to three biopsies of muscle tissue were obtained from each subject during the procedure and then immediately microdissected free of any fat or connective tissue at 4°C. Aliquots were immediately snap frozen in liquid nitrogen and stored at −80°C for subsequent morphological and molecular studies. The majority of material was, however, prepared for isolation of viable proliferating skeletal muscle satellite cells. We optimized techniques for the isolation of viable satellite cells and the dispersal and proliferation of skeletal muscle cells from them using significantly smaller biopsies than has previously been reported (31).

The establishment and proliferation of human skeletal muscle cells, which exist either as mononuclear myoblasts or as fused multinuclear myotubes, was performed by modification of previously described methods (32). Prior ethical committee approval was obtained. After excision, biopsy material was transferred to transport medium (Hams F10 with 20% FCS, 1,000 units/ml penicillin, 50 μg/ml streptomycin, and 1 μg/ml amphotericine B), which was maintained at 4°C. Fat and connective tissue was carefully microdissected and the remaining muscle finely chopped with scissors. Tissue minces were washed with ice-cold serum-free medium (three times) and, after resuspension in prewarmed sterile-cell dispersal solution (0.05% trypsin and 0.05 mol/l Na EDTA in PBS), transferred to a sterile conical flask and horizontally shaken at 190 rpm for 60 min at 37°C. Tissue debris was allowed to settle for 1 min, the supernatant was centrifuged (550*g*, 2 min at room temperature), and the isolated cell pellet was resuspended in cell growth medium (Dulbecco's Modified Eagle's Medium [DMEM] with 0.11 g/l sodium pyruvate, 200 units/ml penicillin, 50 μg/ml streptomycin, 0.3 mg/ml L-glutamine, 0.25 μg/ml amphotericin B, and 20% FCS). The cells were washed twice in cell growth medium and plated onto gelatin/fibronectin-coated 10-cm dishes in growth medium supplemented with 1% chick embryo extract and 10–25% conditioned medium from highly proliferating myoblasts and placed in a humidified 95% air/5% CO_2 atmosphere at 37°C (Biohit; Wolf Laboratories, Pockington, York, U.K.). Culture media was changed twice weekly but involved removal and replacement of only three-quarters of the total on each occasion. The period to 95% confluency was largely dependent on the yield of viable satellite cells but varied between 6 and 9 weeks for initially plated cells. It is the satellite cells

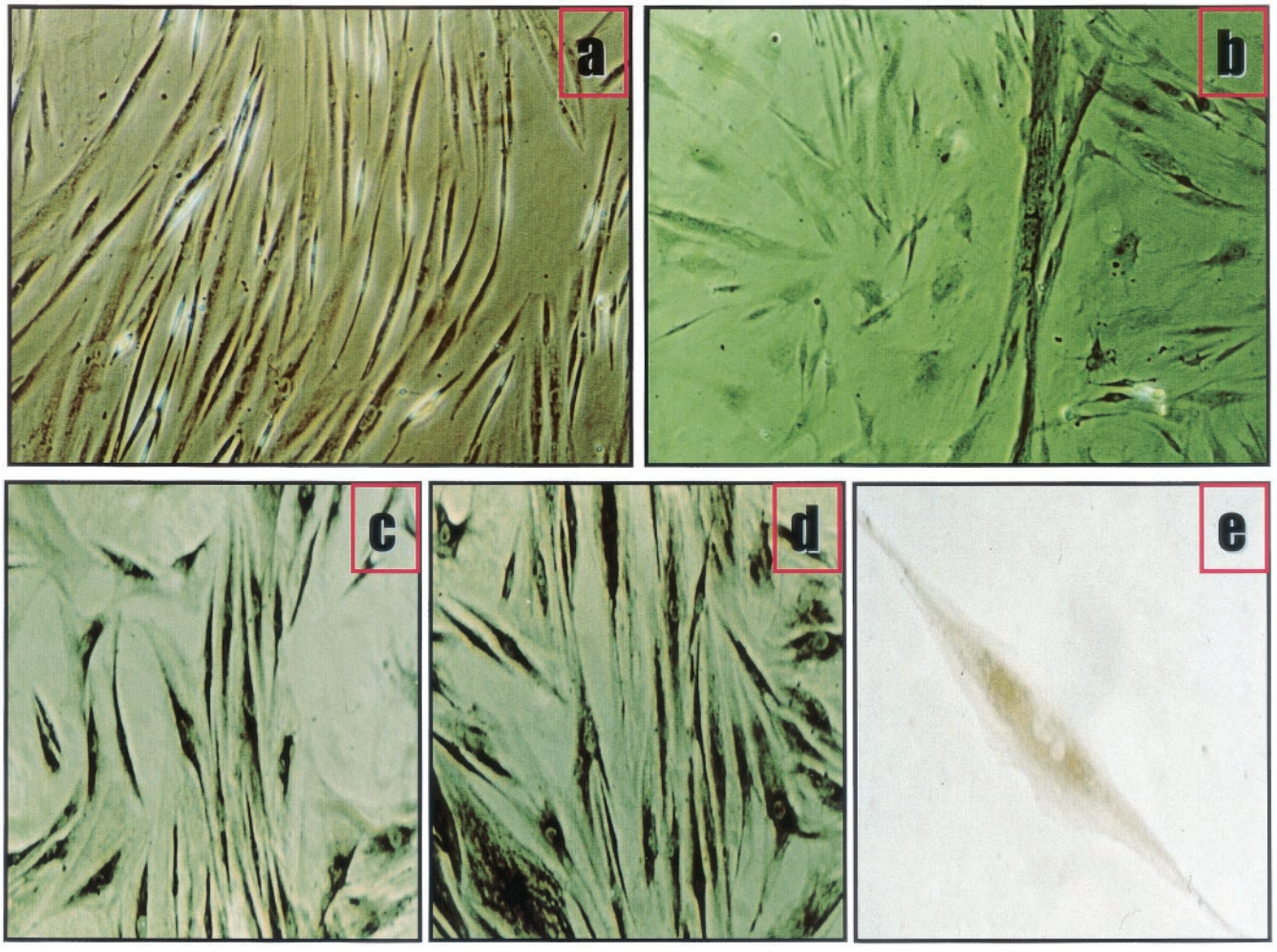


FIG. 1. Morphological analyses of human skeletal myoblasts (*A*) and spontaneous induction of myoblast fusion into multinucleate myotubes, indicated by the arrow (*B*). Immunohistochemical characterization of skeletal myoblast phenotype was confirmed by positive staining for sarcomeric-α-actinin (*C*) and negative staining for human fibroblast surface antigen (data not shown, but signal equivalent to that of unstained cells shown in *A* and *B*). Immunohistochemical analyses of human GR (*D*) and 11β-HSD1 (*E*) expression revealed both nuclear and cytosolic staining for GR in all cells in each flask cultured in the absence of glucocorticoid. Expression of 11β-HSD1 was similarly detected in all cells within each flask (data not shown). A higher magnification photomicrograph revealed that in contrast with the GR, 11β-HSD1 immunostaining within each cell appeared to be confined predominantly to the cytosol (*E*). *A*, *D*, and *E* as previously reported (31).

that retain the capacity for proliferation in culture. They reside between the sarcoplasmic reticulum and the basement membrane and represent a small fraction of the biopsy material itself. As such, the pooling of tissue minces from the two to three samples obtained from each subject, coupled with the optimization of cell isolation protocols, maximized the yield of viable satellite cells from each very-small aliquot of total biopsy material (15–50 mg). Experimental analyses were performed on 95% confluent cells between passages 3 and 12. Isolated cells were >99% skeletal myoblasts, as confirmed by morphological and immunohistochemical analyses.

Immunohistochemical analyses. Cells were washed with PBS, air dried, and fixed with either 10% formol saline or 4% paraformaldehyde. Five to six circles (20-mm diameter) per dish of cells were marked out using a hydrophobic resin to allow multiple parallel immunostaining with various antisera. Nonspecific immunostaining was diminished by incubation with blocking solution (5% serum from the species in which the secondary antisera was raised; obtained from Dako) in PBS for 1 h at room temperature, followed by washing with PBS (three times), and incubation with primary antisera for human GR at 1:100 to 1:1,000 (affinity-purified rabbit polyclonal antibody was raised against a 16–amino acid peptide corresponding to the NH_2-terminus of GR that is common to both the 95-kDa GRα and -β isoforms, obtained from Santa Cruz Biotechnology, Santa Cruz, CA), skeletal muscle desmin antisera at 1:10 to 1:100, mouse monoclonal sarcomeric α-actinin antisera at 1:100 to 1:1,000, mouse monoclonal anti-human fibroblast surface protein antisera at 1:500 to 1:2,000, and mouse monoclonal anti α-smooth muscle actin at 1:500 to 1:2,000 for either 2 h at room temperature or overnight at 4°C in a humidified box, as previously described (32). Working dilutions of antisera were prepared using PBS/0.05% Tween 20 (PBST). Omission of primary antisera, absorption of primary antisera with appropriate purified proteins, and use of nonimmune sera (Dako A/S Denmark) served as negative controls of specific immunostaining (data not shown). After further washing with PBS (three times), cells were incubated with horseradish peroxidase–conjugated anti-rabbit IgG or anti-goat IgM secondary antisera at 1:1,000 to 1:2,500 for a further 1 h at room temperature, and immunostaining was detected using a brief incubation with diaminobenzadine and visualized under light microscopy (Fig. 1).

Enzyme activities. Triplicate 25-cm^2 flasks of subconfluent cells were incubated with 5 ml DMEM containing insulin (20–100 μU/ml), cortisol (50–1,000 nmol/l), and cortisone (50–1,000 nmol/l), separately and in combination, for 48–96 h before assay of 11β-HSD activity. Cells were washed (three times) in hormone-free DMEM and incubated with 200 nmol/l cortisol for 24–48 h. Kinetic analyses of 11-oxoreductase and 11-dehydrogenase activities were conducted, with concentrations of cortisone or cortisol in the range of 31.25–1,000 nmol/l, for a period of 24–48 h. Aliquots of culture medium from each flask, before and after incubation with substrates for 11β-HSD activity, were removed for assay of cortisol and cortisone by high-performance liquid chromatography (HPLC). Briefly, steroids were quantitatively extracted from 4 ml culture medium (containing 80 μg dexamethasone as internal standard for the HPLC) through preconditioned Sep-Pak plus C^{18} cartridges (Millipore U.K./Waters Chromatography Division, Watford, Hertfordshire, U.K.). The steroids were eluted using 5 ml ethylacetate:diethylether (4:1) and washed with 2 ml of 1 mol/l NaOH saturated with Na_2SO_4 followed by 2 ml of 1% acetic acid saturated with Na_2SO_4. The phases were separated, and the aqueous layer was discarded. The remaining organic layer was evaporated to dryness under

a stream of dry nitrogen. The residue was reconstituted with 240 μl of 20% acetonitrile/H_2O, and 160 μl of this was injected onto a Waters Nova-Pak 60 angstrom 30-cm C^{18} reverse-phase HPLC column (Millipore U.K./Waters Chromatography Division). After programmed gradient elution with mobile phases that comprised Phase A (50 mmol/l KH_2PO_4 and 10 mmol/l acetic acid) and Phase B (65% acetonitile in Phase A) at a flow rate of 0.8 ml/min, the steroids were detected by ultraviolet absorbance at 247 nm. Cortisol and cortisone levels were quantified against known internal standards.

Although the maximum absorbance for steroids with an α,β-unsaturated ketone in the A-ring can typically be found at 240 nm, the absorbance maximum for both cortisol and cortisone using this technique (using several scanning UV spectrophotometers) was 247 nmol/l. This technique has been fully validated against fluorescence detection and GC-MS (S.J.D., unpublished observations). The limits of detection for this technique were estimated to be 1.6 ± 0.8 nmol/l (i.e., ~2.4 ng steroid injected onto the column) for all steroids examined. Mean analytical recovery for cortisol and cortisone were 98.9 ± 2.5% and 96.5 ± 3.0%, respectively. Analytical imprecision (coefficient of variation [CV] %) was estimated to be 6.1 ± 1.2% (within batch) and 8.3 ± 2.4% (between batch) for a range of typical in vivo cortisol and cortisone concentrations.

In addition to cortisol and cortisone, this technique was capable of detecting all steroids, with an unsaturated ring A, and was validated for the quantitative estimation of 6β-hydroxycortsol, 20α-dihydrocortisol, 20β-dihydrocortisol, 20α-dihydrocortisone, 20β-dihydrocortisone, cortisol, cortisone, dexamethasone, corticosterone, 11-deoxycortisol, 11-deoxycorticosterone, 17α-hydroxyprogesterone, androstenedione, and progesterone. Although the more polar tetrahydrometabolites of cortisol and cortisone would not be detected by this technique, it is highly unlikely that this degree of metabolism (more akin to hepatic than peripheral metabolism of glucocorticoids) would occur in human skeletal myoblasts. Importantly, the sum of cortisol and cortisone that was quantitatively extracted from the cell culture medium subsequent to incubation was not significantly different from that which had been added before incubation (within the aforementioned limits of experimental error and methodological imprecision). This is consistent with there being undetectable levels of either cortisol or cortisone in cell culture medium containing FCS. Because 11β-HSD1 behaved exclusively as an 11-oxoreductase in these cells, any cortisol present in the cell culture medium remained unmetabolized. This was confirmed by assay of cortisol concentrations after incubation of cells with cortisol during assays of 11-dehydrogenase activity.

To maintain first-order kinetics for the analysis of 11β-HSD1 activity in these cells, substrate (i.e., cortisone) concentrations and sampling times were established so that reaction rates were well within the linear part of the reaction velocity versus substrate concentration plot, as we have previously described (33).

Western blot. Cells were washed with ice-cold PBS (three times), gently scraped from flasks, briefly centrifuged (750*g*, 2 min), and suspended in either PBS/1 mmol/l PMSF (phenylmethylsulfonyl fluoride) (for total cell protein) or NP40 lysis buffer (0.05% NP40 in phosphate buffer, pH 7.2) and subjected to differential centrifugation to enable isolation of nuclear and cytosolic protein. Varying concentrations of protein, assayed by Bradford method using a commercially available kit (Biorad Laboratories, Herts, U.K.), were mixed with one volume of SDS-glycerol/β-mercaptoethanol/bromophenol blue loading buffer, heated to 95°C for 5 min, and electrophoresed alongside molecular weight markers through 4% stacking and 10–12% resolving denaturing SDS-PAGE gels. After electroblotting (35 $V \cdot 0.8\ mA^{-1} \cdot cm^{-2}$) onto Hybond C membranes (Amersham Pharmacia Biotech, Bucks, U.K.), samples were incubated with blocking solution (10% milk powder/PBS/0.05% Tween 20/1% goat serum) for 1 h at room temperature, followed by incubation with rabbit anti–human GR polyclonal antisera (Santa Cruz Biotechnologies) in 1% milk powder/PBS/0.05%Tween 20 for 3 h at room temperature. After washing with PBS (three times), membranes were incubated with horseradish peroxidase–conjugated goat anti–rabbit IgG at 1:100 for 1 h at room temperature. Human glucocorticoid receptor (hGR) was visualized using the enhanced chemiluminescence plus system (Amersham Pharmacia Biotech), exposed to autoradiograhic film within its linear range, and then the signal was quantified by scanning laser densitometry.

RT-PCR. RNA was isolated from tissue and cultured myocytes using a single-step acidified phenol/chloroform extraction method (RNAzol B; Biogenesis, Poole, U.K.). First-strand DNA was synthesized from 10 μg total RNA using RT-driven primer extension from either random hexamers or 3′-antisense oligonucleotide primers corresponding to human GRα, GRβ, 11β-HSD1, 11β-HSD2, mineralocorticoid receptor (MR), and sodium-potassium adenosine triphosphatase α1 subunit, as we have previously described (34). The upstream primer used for the amplification of GRα and -β mRNA was identical, i.e., 5′-ACTTACACCTGGATGACCAAAT-3′, whereas the downstream primers were specific for either GRα, i.e., 5′-TTCAATACTCATGGTCTTATCC-3′, or GRβ, i.e., 5′-TCCTATAGTTGTCGATGAGCAT-3′. The sequences of the primers used for the detection of the other genes have been previously described (34). Briefly, RNA was heated to 65°C for 5 min, snap cooled to 4°C, mixed with reaction buffer (50 mmol/l Tris-HCl, 50 mmol/l KCl, 10 mmol/l $MgCl_2$, 10 mmol/l DTT, and 0.5 mmol/l spermidine, pH 8.3), ribonuclease inhibitor (RNAsin; Promega, Southampton, Hampshire, U.K.), dNTPs (10 mmol/l each of dATP, dCTP, dGTP, and dTTP), 30 pmol/l primers, and 200 U Superscript II (Amersham Pharmacia Biotech) in DEPC (diethyl pyrocarbonate)-treated water at a volume of 50 μl, and incubated at 42°C for 1 h. Five to ten percent of this reaction served as a template for the PCR amplification of fragments of these mRNAs using specific sense and antisense primers to generate DNA products of the predicted sizes shown in Table 2. cDNAs, including pT7/T3 hGRα cDNA fragment, pT7/T3 hGRβ cDNA fragment, pT7/T3-hGRα full-length cDNA, 11β-HSD1/pcDNAI, h11β-HSD2/pGEM4Z, hMR cDNA, and hNa/K ATPase α1 cDNA and RT products from previous assays served as positive controls for the PCR step. A total RNA pool served as positive controls for the RT step. Negative controls included PCR of non–reverse-transcribed RNA samples and omission of primers from both RT and PCRs. RT-PCR products were electrophoretically fractionated on 1% ethidium bromide–stained agarose gels.

Northern blot. Total RNA (30–50 μg/lane) from tissue and cultured myocytes was electrophoresed alongside RNA molecular weight markers (Amersham Pharmacia Biotech) in a 1.5% agarose/15% formaldehyde/1× MOPS gel at 100 mA for 4–6 h, followed by transfer to Hybond N+ membranes. Parallel dot-blot analyses of each RNA preparation were also performed using a Hybridot apparatus (Gibco-Life Technologies) to assist quantification of mRNA and recorded as arbitrary units in relation to those for 18S rRNA by scanning laser densitometry. Membranes were hybridized with either cDNA or cRNA probes for GR, 11β-HSD1, and 18S in either 0.77 mol/l sodium phosphate/5 mmol/l EDTA/7% SDS/200 μg/ml denatured salmon sperm DNA (ssDNA) buffer (pH 7.2) at 65°C for cDNA probes or 50% deionized fomamide/2× SSPE/5× Denhardt's solution/10% dextran sulfate/0.1% SDS and 200 μg/ml ssDNA buffer at 42°C for cDNA probes or 63°C for cRNA probes. Membranes were washed in 2 × sodium chloride–sodium citrate (SSC) (1 × SSC = 150 mmol/l NaCl/15 mmol/l trisodium citrate) − 0.1% SDS (10 min at room temperature) and up to a maximum stringency of 0.1 × SSC − 0.1% SDS (30 min at 68°C). Hybridization signals were analyzed using a Storm 850 phosphorimager (Molecular Dynamics, Piscataway, NJ) and were exposed to autoradiographic film (DuPont-Cronex) between intensifying screens at −70°C for 1–10 days, such that the signal fell within the linear range of the film. Before rehybridization with other probes, cDNA probes were removed from membranes by washing with 1% SDS (3 h at 70°C) and cRNA, and then 18S rDNA probes were removed by washing with 0.1% SDS at room temperature.

Nucleic acid probes. cDNA probes for hGR (35), h11β-HSD1 (36), and ribosomal 18S (37) were radiolabelled with [^{32}P]deoxy-CTP (3,000 Ci/mmol) by random priming of the excised cDNA fragment using commercially available kits (Amersham Pharmacia Biotech). To improve sensitivity, antisense GR cRNA probes were synthesized by in vitro transcription from pT7/T3-hGRa cDNA by T3 RNA polymerase after linearization with *Kpn*1 (unable to distinguish between GRα and -β mRNA) as previously described (25,38). Shorter antisense cRNA probes (~0.6 kb) for the detection of total hGR were also generated from the pT7/T3-hGRα cDNA by T3 RNA polymerase after linearization with *Ssp*I. GR isoform–specific probes were also synthesized as previously described (25,38) from the 537 bp *Pst*I/*Kpn*I fragment pT7/T3-α cDNA by T7 RNA polymerase after linearization with *Pst*I (to detect GRα mRNA alone) and from the 960bp *Pst*I/*Sst*I fragment pT7/T3-β cDNA using T7 RNA polymerase. After linearization with *Nsi*I, a 581-bp probe was generated (to detect GRβ mRNA alone). Antisense h11β-HSD1 cRNA probes (1.2 kb) were generated from the full-length h11β-HSD1 cDNA (36) and subcloned into pBluescript KS+ using T3 RNA polymerase after linearization with *Hind*III. All cRNA probes were radiolabelled by incorporation of [^{32}P]UTP (3,000 Ci/mmol), and synthesis of >90% full-length cRNA probes was confirmed by autoradiography of probes subjected to denaturing (7 mol/l urea) polyacrylamide (6%) gel electrophoresis.

The hGR cDNAs were kindly provided by Drs. Robert Oakley and John Cidlowski (National Institutes of Health, Bethesda, MD), the h11β-HSD1 cDNA was from Professor Perrin White (University of Texas Southwestern Medical Center, Dallas, TX), and the r18S rDNA was from Professor Ira Wool (University of Chicago).

Statistical analyses. Kolmogorov-Smirnov analysis indicated that the data were normally distributed. All data presented are expressed as means ± SE. Data were analyzed using an unpaired Student's *t* test. The significance of linear regression analyses was analyzed by Pearson correlation. Each muscle cell experiment or analysis was performed in triplicate with SEs maintained at <10% of the mean. Where appropriate, *f* test analysis was performed to

determine the statistical difference between two slopes during kinetic analyses. Values were considered significant at $P < 0.05$.

RESULTS

Morphology and immunohistochemical characterization of human skeletal muscle cells. Cell isolation and growth protocols were optimized to yield myoblasts of pure muscle origin (Fig. 1*A*). Serum deprivation induced the formation of multinuclear myotubes (Fig. 1*B*). Induction of myotubes, either spontaneously or after serum deprivation, coupled with positive immunohistochemical staining for skeletal muscle desmin and sarcomeric-α-actinin (Fig. 1*C*) and negative staining for human fibroblast surface protein and α-smooth muscle actin, confirmed yield and proliferation of >99% human skeletal myoblasts, as previously described (31,32). All subsequent analyses were performed on 95% confluent cells that were exclusively myoblasts. Immunohistochemical analyses of GR (Fig. 1*D*) and 11β-HSD1 (Fig. 1*E*) in confluent and subconfluent flasks of human skeletal muscle cells revealed expression of these genes in all cells, as we have previously described (31).

GR expression appeared to be distributed in both nuclear and cytosolic compartments, despite the absence of glucocorticoid in culture medium containing 20% FCS (Fig. 1*D*). Although 11β-HSD1 protein was similarly detected in every muscle cell (data not shown), a higher magnification photomicrograph of a single skeletal muscle cell shows that 11β-HSD1 expression, in contrast with that for the GR, was predominantly confined to the cytosol (Fig. 1*E*). This was confirmed by Western blot analyses of GR and 11β-HSD1 expression in nuclear and cytosolic protein fractions isolated by differential centrifugation (data not shown).

Characterization of glucocorticoid hormone signaling in cultured myoblasts and muscle tissue

Glucocorticoid receptor expression. Analyses of gene expression by RT-PCR using primers specific for GRα, GRβ, MR, and constitutively expressed Na/K-ATPase α1 subunit (across a range of PCR cycle numbers) revealed expression of GRα and -β (Fig. 2*A*) but did not reveal MR mRNA in skeletal muscle biopsies (data not shown). Similar patterns of expression were evident in cultured myoblasts under basal glucocorticoid-free conditions, with the exception that GRβ mRNA was not expressed in myoblasts from any of the subjects (Fig. 2*A*). The detection of GRβ mRNA in cells cultured in the presence of >100 nmol/l cortisol suggests that GRβ expression in skeletal myoblasts may be upregulated by glucocorticoid (39). This necessitated the use of GRα-specific cRNA probes for quantitative analyses of the expression of the ligand-binding GRα variant. Northern blot analyses indicated abundant expression of GRα mRNA that predominantly comprised a 7.0-kb species in skeletal myoblasts (Fig. 2*B*). Detection of 95 kDa GR protein by Western blot using polyclonal GR antisera confirmed translation of GR mRNA in these cells (Fig. 2*B*). Importantly, there was close agreement between GRα mRNA and GR protein levels in analyses of GR expression.

Incubation of cultured myoblasts isolated from a representative subject with increasing concentrations of cortisol resulted in a marked dose-dependent decline in both GRα mRNA and GR protein expression (Fig. 2*B*). This downregulation of GRα by cortisol was abolished by co-incubation with 10-fold molar excess of the GRα antagonist, RU38486 (data not shown). These data suggest variability in basal expression of GRα; its regulation by cortisol in human skeletal myoblasts occurs predominantly at the transcriptional level and is exclusively mediated by binding of cortisol to its receptor. Importantly, incubation of myoblasts with varying concentrations of insulin, IGF-1, and glucose had no significant effect on GRα expression.

11β-HSD expression. Analyses of gene expression by RT-PCR using primers specific for 11β-HSD1 and -HSD2 (across a range of PCR cycle numbers) revealed expression of 11β-HSD1 but not -HSD2 mRNA in skeletal muscle biopsies and cultured myoblasts that were established from them (data not shown). Northern blot analyses of RNA isolated from human skeletal myoblasts revealed abundant expression of 1.4 kb 11β-HSD1 mRNA (Fig. 2*C*). Kinetic analyses revealed that this encodes low-affinity 11-oxoreductase activity with a K_m for cortisone of ~0.5 μmol/l (Fig. 2*D*). Importantly, 11-dehydrogenase activity was undetectable in intact cells. Preincubation of myoblasts with physiological concentrations of cortisol for up to 96 h resulted in a significant dose-dependent increase in 11β-HSD1 mRNA expression at cortisol concentrations >0.1 μmol/l (Fig. 2*C*). In keeping with the gene expression data (Fig. 2*C*), kinetic analyses of enzyme activity (represented by the Hanes plots shown in Fig. 2*D*) indicated that preincubation of cells with 0.5 μmol/l cortisol had no significant effect on the affinity of 11β-HSD1 for cortisone but markedly increased the V_{max} of the 11-oxoreductase activity ($P < 0.001$). This was deduced from *f* analysis of the statistical significance of the difference between the two slopes shown in the Hanes plot (Fig. 2*D*). Analyses of 11-oxoreductase activity using Eadie-Hofstee plots (data not shown) revealed almost identical V_{max} and K_m values as those obtained by Hanes plot analysis. This further supports the validity of the observations of the marked response in 11-oxoreductase activity to treatment by glucocorticoid.

No 11-dehydrogenase activity was detectable, either under basal glucocorticoid–free conditions or after preincubation with cortisol. Consequently, the cortisol-induced increase in 11β-HSD1 mRNA expression resulted exclusively in increased 11-oxoreductase activity–mediated intracellular synthesis of active cortisol from cortisone. Furthermore, the sensitivity of 11β-HSD1 response to cortisol would not have been confounded by potential conversion of cortisol to inactive cortisone. Auto-upregulation of 11β-HSD1 mRNA and 11-oxoreductase activity by cortisol was abolished by co-incubation with a 10-fold molar excess of RU38486 (data not shown), indicating that this effect is mediated exclusively by GRα.

Importantly, between-subject differences in levels of GR and 11β-HSD1 mRNA expression remained unchanged, irrespective of cell passage number (passages 3–12) or cell density (up to 98% confluence, i.e., 2.9×10^6 cells/25-cm^2 flask), either during basal conditions or after incubation with glucocorticoid. Thus, variability attributable to potentially important sources of methodological error was minor, particularly when compared with the marked be-

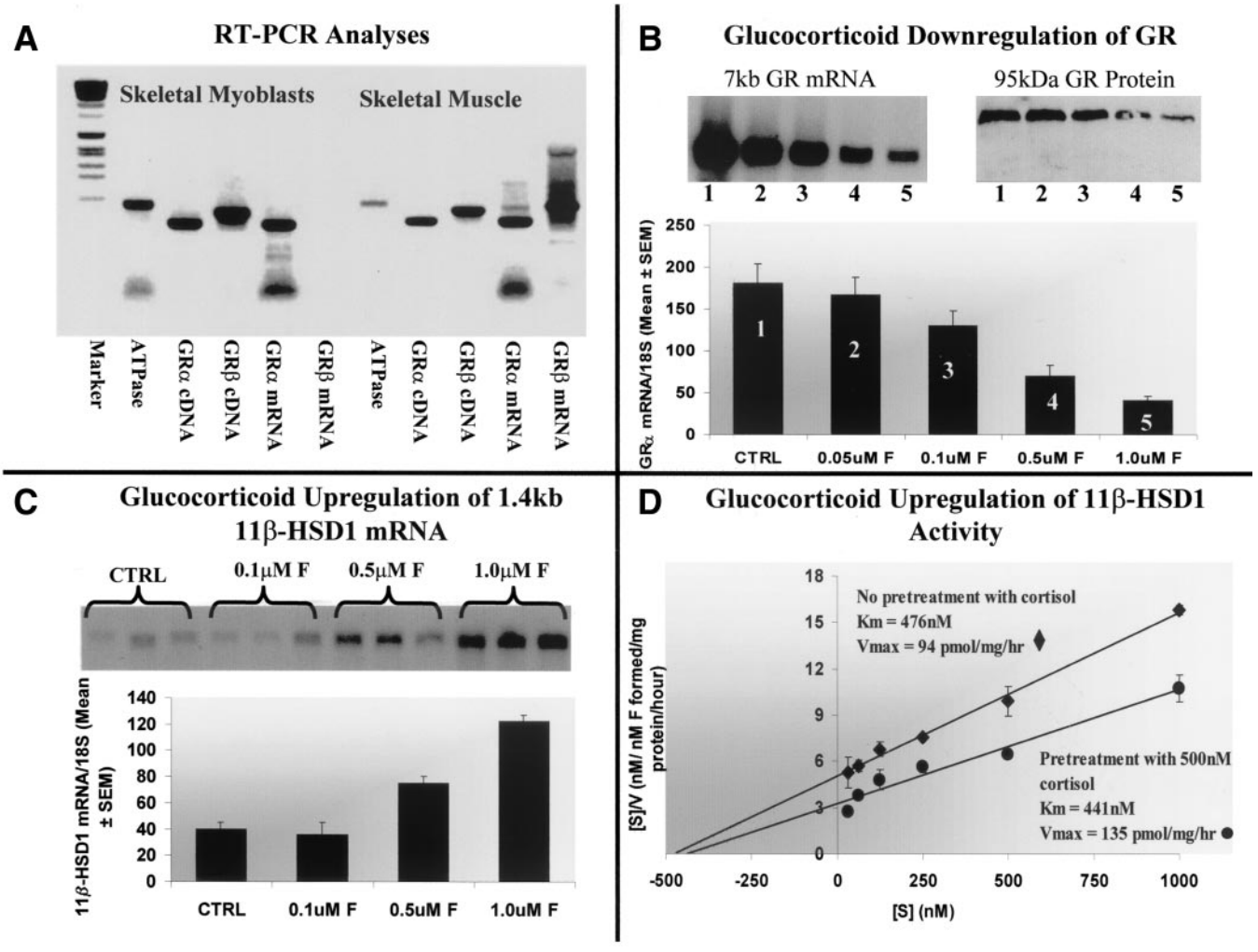
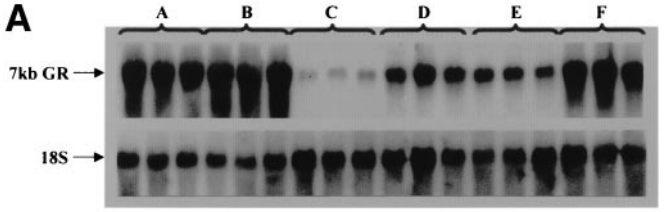


FIG. 2. Characterization of GRα and 11β-HSD1 expression and their regulation by glucocorticoid in myoblasts from a representative subject. *A*: RT-PCR analyses of GRα and -β mRNA expression in cultured skeletal myoblasts and representative skeletal muscle tissue. GRα and GRβ cDNAs served as positive controls for the PCR and Na/K-ATPase as a positive control for the integrity of the RNA in each sample. *B*: Northern blot analysis of GRα mRNA and Western blot analysis of GR protein expression in myoblasts under basal conditions and after 48 h incubation with physiological concentrations of cortisol. *C*: 11β-HSD1 mRNA expression in myoblasts cultured under basal conditions and after physiological concentrations of cortisol. *D*: Kinetic analyses (Hanes plots) of 11β-HSD1 11-oxoreductase activity in myoblasts cultured in the absence of glucocorticoid and after 48 h preincubation with 0.5 umol/l cortisol. [S], substrate concentration; V, rate of reaction expressed as pmol/l cortisol formed · mg protein^{-1} · h^{-1}. Each data point reflects the mean ± SE of the reaction rate at each substrate concentration measured across triplicate experiments. Because 11-oxoreductase activity was measured by the accumulation of cortisol over a 24-h period in the absence of the original glucocorticoid stimulus, the 40% increase in V_{max} is likely to be an underestimate of that occurring within the first few hours of the incubation with the substrate, cortisone. A significant decline in 11β-HSD1 mRNA levels observed within 12 h of the withdrawal of glucocorticoid ($P < 0.01$, data not shown) is consistent with this hypothesis.

tween-subject differences for both GRα (as shown in Figs. 3*A* and *B*, 4*A*, and 5*A*) and 11β-HSD1 (Figs. 3*B* and 7*A*).

Associations between GR expression and features of the metabolic syndrome. Northern blot analyses revealed marked between-subject differences in myoblast GRα mRNA expression under basal glucocorticoid-free conditions (Fig. 4). Parallel Western blot analyses also revealed marked between-subject differences in GR protein expression that closely agreed with levels of GRα mRNA ($r^2 = 0.84$, $P < 0.001$). A significant inverse correlation was present between myoblast GRα expression in vitro and glucose disposal rate in vivo (Fig. 4*B*). In addition, positive associations were present between myoblast GRα expression and BMI (Fig. 4*C*), percent body fat (Fig. 4*D*), and systolic blood pressure (Fig. 4*E*). These associations persisted when myoblasts were cultured in the absence of serum (data not shown).

Because levels of GRα expression are dependent on the prevailing concentrations of glucocorticoid (Fig. 2*B*), we repeated these analyses after preincubation of myoblasts with 0.2 μmol/l cortisol for 48 h. Importantly, under these conditions, the relationships between skeletal myoblast GRα expression and in vivo glucose disposal rate (Fig. 5*B*), BMI (Fig. 5*C*), percent body fat (Fig. 5*D*), and systolic blood pressure (Fig. 5*E*) were similar to those observed under basal glucocorticoid-free conditions. There was a strong correlation ($r^2 = 0.87$, $P < 0.001$) between GRα expression under basal conditions and after exposure to physiological concentrations of cortisol. Although glucocorticoid-dependent inhibition of GRα expression occurred across all subjects, myoblasts with higher basal levels of GRα expression also had higher levels of GRα expression after exposure to cortisol (Fig. 6*A*). No statistically significant associations were evident between muscle cell expression of GRα and the age of the subjects from whom the cells originated.



B

Intrasubject variability	%CV	*n*
Intraflask variation	<5	6
Temporal variation (9 subjects)	8.7–19.7	6
Intra-Northern blot variation	10.1–14.0	6
Inter-Northern blot variation	<13.6	4
Interpassage variation (4 subjects)	12.7–22.5	5
Variation due to cell density (4 passages)	9.4–16.1	12

FIG. 3. *A*: Representative Northern blot analysis of GRα mRNA expression in skeletal myoblasts from six subjects (*A–F*), with contrasting levels of insulin resistance, blood pressure, and adiposity. RNA in each lane is pooled from three flasks of cells for one cell passage, such that GRα expression in myoblasts from three different passages for each subject is shown. For each subject, *lane 1* = passage 3, *lane 2* = passage 7, and *lane 3* = passage 10. *B*: Summary of percent CV analyses for variations in GRα expression that may be due to methodology. As previously reported (31).

Associations between 11β-HSD1 expression and features of the metabolic syndrome. Marked differences in levels of 11β-HSD1 mRNA and parallel levels of 11-oxoreductase activity were observed among subjects. In contrast with GRα expression, no significant associations were observed between 11β-HSD1 mRNA expression or 11-oxoreductase activity in skeletal myoblasts and any of the features of the metabolic syndrome under basal glucocorticoid-free conditions. However, after incubation with physiological concentrations of glucocorticoid, which resulted in GRα-mediated parallel increases in 11β-HSD1 mRNA expression and 11-oxoreductase activity (Figs. 2*C* and *D* and 6*B*), a strong inverse correlation was evident between myoblast 11β-HSD1 expression and in vivo glucose disposal rate (Fig. 7*B*). Moreover, positive associations were also present between myoblast 11β-HSD1 and BMI (Fig. 7*C*) and systolic blood pressure (Fig. 7*E*). The close agreement between levels of 11β-HSD1 mRNA and 11-oxoreductase activity in these cells in response to treatment with glucocorticoid (exemplified by data shown in Fig. 2*D*) and other factors (39) was consistent with similar associations with regard to enzyme activity (data not shown).

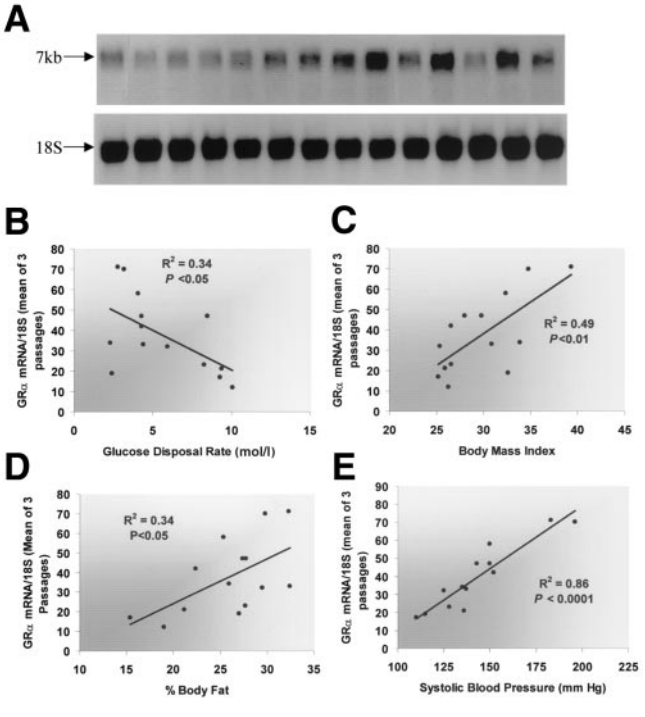


FIG. 4. Associations between skeletal myoblast GRα expression under basal glucocorticoid-free conditions and in vivo insulin sensitivity, adiposity, and systolic blood pressure in lean, moderately overweight, and obese men. *A*: Representative Northern blot analysis of GRα mRNA expression in myoblasts from subjects with lower (left) and higher (right) glucose disposal rate. *B– E*: Linear regression analyses depicting significant associations between constitutive GRα expression and glucose disposal rate (*B*), BMI (*C*), percent body fat (*D*), and systolic blood pressure (*E*). Each data point used in these analyses represents expression in RNA pooled from three flasks from each of three different passages. The systolic blood pressure for one of the subjects was >2 SDs above the arithmetic mean for the whole subject group and was therefore excluded from regression analyses relating to blood pressure.

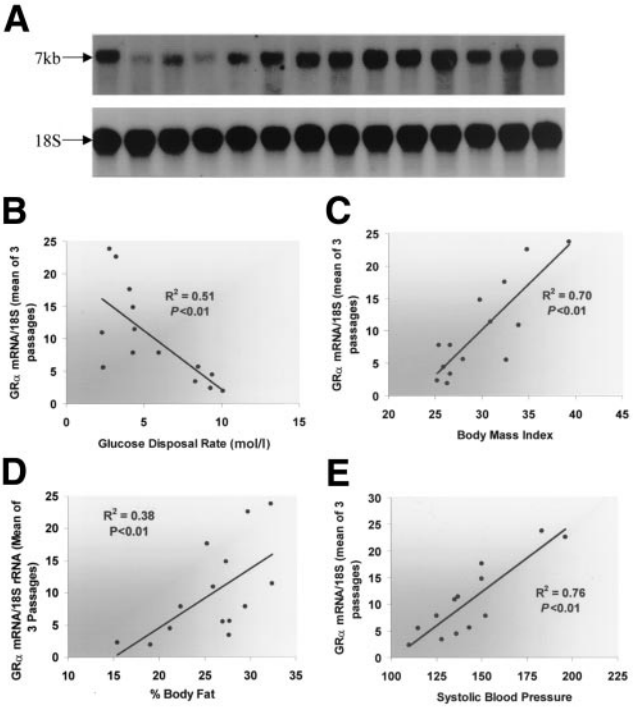


FIG. 5. Associations between GRα expression in skeletal myoblast after incubation with 200 nmol/l cortisol and in vivo insulin sensitivity, adiposity, and systolic blood pressure in lean, moderately overweight, and obese men. *A*: Representative Northern blot analysis of GRα mRNA expression in myoblasts from subjects with lower (left) and higher (right) glucose disposal rate. *B–E*: Linear regression analyses depicting significant associations between constitutive GRα expression and glucose disposal rate (*B*), BMI (*C*), percent body fat (*D*), and systolic blood pressure (*E*). Each data point used in these analyses represents expression in RNA pooled from three flasks from each of three different passages. The systolic blood pressure for one of the subjects was >2 SDs above the arithmetic mean for the whole subject group and was therefore excluded from regression analyses relating to blood pressure.

DISCUSSION

We describe for the first time novel data relating to analyses of glucocorticoid hormone signaling in human

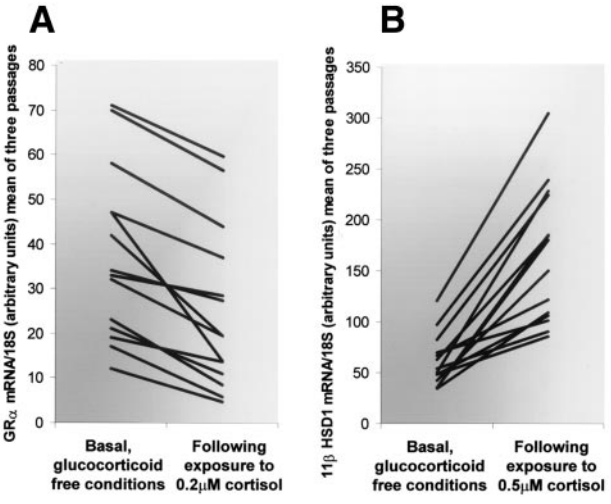


FIG. 6. Comparison of basal and glucocorticoid-induced downregulation of GRα mRNA expression (*A*) and basal and glucocorticoid-induced upregulation of 11β-HSD1 mRNA expression (*B*) in skeletal myoblasts from 14 male subjects with contrasting levels of insulin sensitivity, adiposity, and blood pressure. RNA was pooled from three flasks from each of three different passages for each subject. Levels of GRα mRNA expression under basal glucocorticoid-free conditions and after exposure to 0.2 μmol/l cortisol (approximating physiological levels) were inversely associated with glucose disposal rate (r^2 = 0.34 and 0.51, $P < 0.05$ and <0.01, respectively) and positively associated with BMI (r^2 = 0.49 and 0.70, $P < 0.01$), body fat (r^2 = 0.34 and 0.38, $P < 0.05$ and <0.01), and systolic blood pressure (r^2 = 0.86 and 0.76, $P < 0.0001$ and <0.01). Levels of 11β-HSD1 mRNA expression after exposure to 0.5 μmol/l cortisol (representing the K_m for this enzyme) were inversely associated with glucose disposal rate (r^2 = 0.68, $P < 0.0001$) and positively associated with BMI (r^2 = 0.63, $P < 0.005$) and systolic blood pressure (r^2 = 0.27, $P < 0.05$). Under basal glucocorticoid-free conditions, no associations were observed between 11β-HSD1 mRNA expression and glucose disposal rate, BMI, or systolic blood pressure.

skeletal myoblasts. Importantly, these results reveal key molecular mechanisms underlying recent suggestions that altered levels of glucocorticoid hormone action may play an important role in the etiology of the metabolic syndrome (12). Our analyses indicate important associations between in vivo features of the metabolic syndrome and levels of basal and glucocorticoid-dependent GRα expression and glucocorticoid-dependent 11β-HSD1 expression in skeletal myoblasts in vitro.

Studies investigating mechanisms underlying GR expression and GR downregulation by its ligand, cortisol, indicate that this occurs predominantly at the level of mRNA transcription and stability and, to a much lesser degree, through post-translational increases in GR turnover (23). Thus, most GR regulation studies have focused on analyses of GR mRNA expression (23,40). The close agreement between levels of GRα mRNA and protein evident in our study is consistent with predominantly transcriptional regulation of GR expression in human skeletal myoblasts. Abolition of this GR downregulation by blockade of GRα with RU38486 confirms that this is exclusively mediated by the ligand-binding GRα. Importantly, although GRα downregulation by cortisol is evident in myoblasts from all subjects, the strong association between basal levels of GRα mRNA expression and GRα mRNA expression after exposure to cortisol and their associations with glucose disposal rate, BMI, and systolic blood pressure suggest that at physiological cortisol concentrations, myoblast expression of GRα is generally greater in individuals with key features of the metabolic syndrome.

GRα exposure to intracellular cortisol is largely regulated by isoforms of 11β-HSD, which mediate interconversion between cortisol and inactive cortisone (24). Recent studies suggest that 11β-HSD1–mediated regulation of intracellular conversion of cortisone to cortisol plays a key role in the etiology of insulin resistance (12,28), central obesity (27), and hypertension (26). Pharmacological 11β-HSD1 inhibition experiments in humans (28), coupled with 11β-HSD1 gene knockout studies (30), suggest that reduced hepatic 11β-HSD1 activity may increase insulin sensitivity regardless of circulating glucocorticoid levels. 11β-HSD1 11-oxoreductase activity may, therefore, serve to maintain intracellular cortisol concentrations at higher levels than those present in the circulation. We have detected abundant levels of 11β-HSD1 gene expression in cultured human skeletal myoblasts and demonstrated that it behaves exclusively as an 11-oxoreductase, with rates of activity similar to those reported in other isolated human cells (27,33,40). Unlike GRα expression, basal levels of 11β-HSD1 expression in skeletal myoblasts are not associated with features of the metabolic syndrome.

We have demonstrated that 11-oxoreductase activity in human skeletal myoblasts is sensitively upregulated by

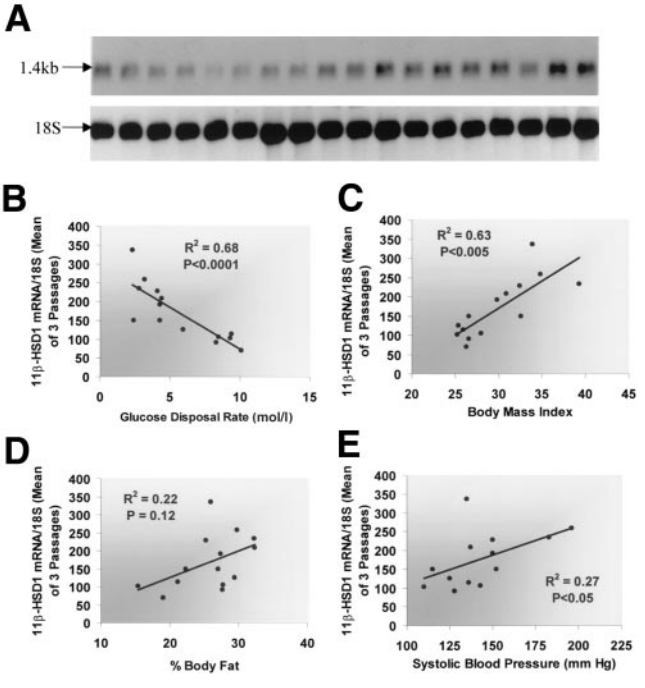


FIG. 7. Associations between 11β-HSD1 expression in skeletal myoblasts incubated with 500 nmol/l cortisol and in vivo insulin sensitivity, adiposity, and systolic blood pressure in lean, moderately overweight, and obese men. *A*: Northern blot analysis of 11β-HSD1 mRNA expression in myoblasts from five subjects with lower glucose disposal rate (left) and four subjects with higher glucose disposal rate (right). Each of the two lanes of RNA per subject reflect 11β-HSD1 expression in cells from two different passages, with RNA pooled from three flasks for each passage. *B*– *E*: Linear regression analyses depicting significant associations between 11β-HSD1 expression in skeletal myoblasts after preincubation with 500 nmol/l cortisol and glucose disposal rate (*B*), BMI (*C*), percent body fat (*D*), and systolic blood pressure (*E*). Each data point represents expression in RNA pooled from three flasks from each of three different passages. The systolic blood pressure for one of the subjects was >2 SDs above the arithmetic mean for the whole subject group and was therefore excluded from regression analyses relating to blood pressure.

physiological concentrations of cortisol and that this arises from increased levels of 11β-HSD1 mRNA expression. These findings accord with previous studies suggesting that 11β-HSD1 11-oxoreductase activity is increased by glucocorticoid in cultured adipose stromal cells and hepatocytes (27,41). Importantly, in the presence of glucocorticoid, skeletal myoblast expression of 11β-HSD1 (like that for GRα) is inversely correlated with glucose disposal rate and positively associated with BMI and blood pressure in vivo. Abolition of glucocorticoid induction of 11β-HSD1 in human skeletal myoblasts by RU38486 confirms that this effect is mediated exclusively by the ligand-binding GRα variant. As such, it is likely that the magnitude of the 11β-HSD1 response in skeletal muscle cells is directly dependent on basal and glucocorticoid-dependent levels of GRα expression.

Upregulation of 11β-HSD1 11-oxoreductase activity represents a potentially powerful mechanism by which glucocorticoid hormone action may be amplified several fold within the cell. In the absence of limiting factors, this would be predicted to perpetually amplify glucocorticoid hormone action within skeletal muscle and other insulin targets, i.e., fat and liver cells in which auto-upregulation of 11β-HSD1 has been reported (27,41). We report for the first time that a key factor limiting this process in human skeletal muscle cells is the expression of GRα. We propose that GRα expression is likely to be the principal determinant of the equilibrium between 11β-HSD1 and GRα activity, and the balance of this equilibrium dictates the magnitude of overall glucocorticoid hormone action within the skeletal muscle cell.

Our data suggest that the position of equilibrium between 11β-HSD1 and GR activity is markedly altered in skeletal muscle cells from subjects with features of the metabolic syndrome because of altered GRα expression. The position of this equilibrium would maintain much higher intracellular cortisol concentrations (and hence glucocorticoid hormone action) in these subjects. In this respect, the absence of any effects of either insulin or glucose on GRα expression in human skeletal myoblasts supports the hypothesis that maintaining high levels of GRα expression in skeletal muscle cells in vivo may constitute an important fundamental mechanism at the center of the pathophysiology of the metabolic syndrome. Relatively small increases in the expression of GRα may result in disproportionately large increases in glucocorticoid hormone action and thus promote insulin resistance.

Potent upregulation of GRα and/or 11β-HSD1 by proinflammatory cytokines (interleukin-1β and tumor necrosis factor-α) in human skeletal myoblasts (39) and other cell-types (42) may shift the position of the GRα/11β-HSD1 equilibrium further toward increased levels of glucocorticoid hormone action. This suggests a novel mechanism by which elevated levels of these cytokines present in the circulation—fat and skeletal muscle from obese insulin-resistant subjects (43,44) may induce insulin resistance.

It is extremely unlikely that the strong associations between skeletal muscle cell GRα and 11β-HSD1 expression in vitro and features of the metabolic syndrome in vivo are an artifact of cell culture conditions. Our findings imply that expression of GR and 11β-HSD1 in cultured human skeletal muscle cells closely correlates with that in skeletal muscle cells in the body. Crucially, between-subject differences were robustly maintained, irrespective of potential sources of methodological variation such as cell passage or density. We were careful to ensure that skeletal myoblasts were not contaminated by other cell types and confirmed that they exhibited classical characteristics of skeletal muscle cells. Furthermore, immunohistochemical analyses indicate homogeneity in levels of both GRα and 11β-HSD1 expression between cells from each subject. Importantly, previous studies have shown that cultured human skeletal myoblasts also maintain levels of insulin sensitivity that closely correlate with levels of insulin-induced glucose disposal and glycogen synthase activity in vivo (9,10). Thus, the differences in GRα and 11β-HSD1 expression we have observed among individuals are likely to underestimate the importance of these key determinants of tissue sensitivity to glucocorticoid in pathophysiological mechanisms contributing to the etiology of the metabolic syndrome. Elucidation of the mechanisms underlying the important phenomena we have described, whether genetic, epigenetic, or prenatally programmed, requires further investigation.

Circulating levels of cortisol in obese subjects and those with features of the metabolic syndrome have been reported to be normal, reduced, or elevated (15–20) and also exhibit an enhanced response to ACTH stimulation (18). Therefore, the maintenance of high levels of GRα, and consequently of 11β-HSD1 expression in their skeletal myoblasts, irrespective of levels of circulating glucocorticoid, provides a plausible molecular mechanism underlying the key role of glucocorticoid hormone action in the pathogenesis of features of the metabolic syndrome. If similar pathophysiological mechanisms operate in visceral adipose depots, which are rich in GRα and 11β-HSD1 expression (27,45), this may also promote the increased "Cushing's-like" obesity that is frequently associated with the metabolic syndrome (16).

In summary, expression of the ligand-binding GRα in skeletal myoblasts is positively associated with features of the metabolic syndrome. Higher levels of GRα expression in myoblasts from subjects with features of the metabolic syndrome suggest increased sensitivity of their skeletal muscle to circulating glucocorticoid. This is associated with increased GRα-mediated upregulation of 11β-HSD1 and is likely to result in elevated levels of intracellular cortisol. Data from this study, therefore, suggest a role for both GRα and 11β-HSD1 in skeletal muscle in the pathogenesis of the metabolic syndrome.

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