Greater replication and differentiation of preadipocytes in inherited corticosteroid-binding globulin deficiency

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Glucocorticoids are pivotal for adipose tissue development. Am J Physiol Endocrinol Metab 284: E1049–E1054, 2003. First published January 28, 2003; 10.1152/ajpendo.00262.2002.—Glucocorticoids are pivotal for adipose tissue development. Rodent studies suggest that corticosteroid-binding globulin (CBG) modulates glucocorticoid action in adipose tissue. In humans, both genetic CBG deficiency and suppressed CBG concentrations in hyperinsulinemic states are associated with obesity. We hypothesized that CBG deficiency in humans modifies the response of human preadipocytes to glucocorticoids, predisposing them to obesity. We compared normal preadipocytes with subcultured preadipocytes from an individual with the first ever described complete deficiency of CBG due to a homozygous null mutation. CBG-negative preadipocytes proliferated more rapidly and showed greater peroxisome proliferator-activated receptor-γ-mediated differentiation than normal preadipocytes. CBG was not expressed in normal human preadipocytes. Glucocorticoid receptor number and binding characteristics and 11β-hydroxysteroid dehydrogenase activity were similar for CBG-negative and normal preadipocytes. We propose that the increased proliferation and enhanced differentiation of CBG-negative preadipocytes may promote adipose tissue deposition and explain the obesity seen in individuals with genetic CBG deficiency. Furthermore, these observations may be relevant to obesity occurring with suppressed CBG concentrations associated with hyperinsulinemia.

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dent activities of preadipocytes from an individual who is homozygous for the CBG null mutation (34) with those of normal preadipocytes. We compared rates of replication and differentiation capacity of these cells. Upon finding differences in these activities, we studied whether human preadipocytes expressed CBG, and we compared GR characteristics and 11β-hydroxysteroid dehydrogenase (11β-HSD) activity as possible mediators of the differences found.

METHODS

Subjects and sample preparation. Abdominal subcutaneous fat of −1 cm³ was obtained (with informed consent) by biopsy through a 1-cm periumbilical incision from a 57-year-old male (body mass index [BMI] 39.4, waist 125 cm) homozygous for the CBG null mutation. As controls, abdominal subcutaneous adipose tissue samples of −1 cm³ were collected (with informed consent) during elective surgery from Caucasian male subjects (see Table 1 for age, BMI, and waist data). It was important to compare samples from the same gender and site, because there are regional differences in rates of differentiation (1) and 11β-HSD activity (6) and regional and gender differences in GR (16). All subjects provided informed written consent. The Greenslopes Private Hospital Ethics Committee and the Princess Alexandra Hospital Research Ethics Committee provided ethical approval for the work. The normal subjects were not specified.

Samples were transported to the laboratory in Ringer solution and were processed within 15 min by collagenase digestion, as previously described (30). The stromovascular digestion, as previously described (30). The stromovascular cell population, were detached with trypsin-Versene and replated at 1 cells/well (subconfluent) in 96-well plates in SCM for 16–20 h. The wells were washed with PBS, and then SCM, SCM + 10–500 nM cortisol or SCM + 10–500 nM RU-486 was reapplied. After 48 h, preadipocyte cell number was assessed using a formazan colorimetric assay (Promega) as previously described (14). Briefly, the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) was added to each well at a concentration of 200 μg/ml. After incubation at 37 °C for 4 h, absorbance at 490 nm was measured using a Bio-Rad 3500 microplate reader. The MTS proliferation assay was validated by confirming that the formazan absorbance vs. direct cell counts were linear over the range studied.

Characteristics of normal preadipocyte sample donors

<table>
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<th>Sample</th>
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<tr>
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</table>

BMI, body mass index.

Table 1. Characteristics of normal preadipocyte
CBG gene expression. Total RNA was extracted from confluent preadipocytes and stored at −70°C before being reverse-transcribed using the random hexamer priming option of a commercially available kit (SUPERSCRIPT, Life Technologies, Gaithersburg, MD). The integrity of the reverse-transcription step was checked by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RT-PCR. A negative control, with no reverse transcriptase added, was included for each RNA sample. Previously published CBG (24) and GAPDH (8) primer sequences and a second set of CBG primers we designed (sense 5′-GACAAGGGGAAATGAACAC-3′, antisense 5′-GCACAGCTTATTGGACCAC-3′) were obtained commercially. The PCR was performed in a Corbett Research PC-90 microplate thermal sequencer with an annealing temperature of 55°C and 2 mM MgCl₂. 

There were no bands in any preadipocyte lane (Fig. 3). The products of 247 and 296 base pairs, the PCR produced similar low levels of G3PD activity (47 vs. 14, 36, and 68 mU/mg protein).

CBG gene expression. There was no evidence of CBG mRNA in normal human preadipocytes. With use of two different sets of CBG primers with expected PCR products of 247 and 296 base pairs, the PCR produced strong bands in the HepG2-positive control lanes, but there were no bands in any preadipocyte lane (Fig. 3).

GR assessment. Dexamethasone binding as a measure of GR number was assessed using a whole cell-binding assay, as previously described (16). Dexamethasone has minimal affinity for CBG (26). Briefly, 25-cm² flasks of confluent cells were washed with PBS and preincubated in DMEM-Ham’s for 30 min at 37°C. This was followed by a 60-min incubation with one of six serial dilutions of 0.78–25 nM tritiated dexamethasone ([3H]Dex; 70–110 Ci/mmol, Amersham Australia) or 0.78–25 nM [3H]Dex with a 250 times excess of unlabeled dexamethasone (Sigma-Aldrich) in DMEM-Ham’s. After the cells were washed with ice-cold PBS, they were lysed, and an aliquot was taken from each flask to count bound [3H]Dex and for protein determination (3). A Scatchard plot provided the binding characteristics for each sample.

11β-HSD activity. Confluent preadipocytes in 25-cm² flasks were washed with PBS and DMEM-Ham’s F-12, with 500 nM triitated cortisol or cortisone applied in triplicate for a 6-h incubation. The cortisone and cortisol in the media were measured by an improved method of HPLC, as previously reported (22). The cells were washed with PBS and lysed by sonication, and the protein was measured (3) to allow the calculation of cortisol/cortisone interconversion in femtomoles per milligram protein per hour. There were no measurable amounts of cortisol or cortisone in the PBS washes or the cell lysates.

Statistical analysis. Comparisons of replication and G3PD activity used the Student’s t-test (two-tailed). The statistical analyses were performed with the data analysis function of Microsoft Excel, version 5. Statistical significance was defined as P < 0.05.
were 263, 488, and 603 fmol/mg vs. 327–599 fmol/mg for normal preadipocytes (Fig. 5).

11β-HSD activity. Type 1 11β-HSD activity was normal, with cortisone-to-cortisol conversion for the two CBG-negative preadipocyte strains being 14 and 22 fmol·mg protein⁻¹·h⁻¹ vs. normal preadipocytes 10, 10, 17, and 18 fmol·mg⁻¹·h⁻¹ (Fig. 6). There was minimal cortisol-to-cortisone conversion (type 2 11β-HSD activity) in CBG-negative or normal cells.

DISCUSSION

Preadipocytes from an individual with complete CBG deficiency had increased proliferation and enhanced PPARγ-mediated differentiation compared with normal preadipocytes. These characteristics may promote adipose tissue deposition by increasing the number of preadipocytes and their conversion to mature adipocytes. The observations may be relevant to the role of CBG in obesity reported in genetic CBG deficiency (34) and the potential role of suppressed CBG levels in hyperinsulinemic humans (10).

The mechanism of hyperproliferation and accentuated thiazolidinedione-stimulated differentiation in preadipocytes from genetically CBG-deficient individuals is unclear, as CBG gene expression was not detected in cultured human preadipocytes. Furthermore, measures of GR binding and 11β-HSD activity were normal.

CBG may be narrowly expressed in human tissues, with definite tissue expression demonstrated in hepatocytes and in placental syncytiotrophoblasts (24) where CBG may modulate glucocorticoid and progesterone tissue interactions (2). CBG has been reported in rodent adipose tissue by corticosterone binding but not gene expression (11, 12), and this may represent circulating CBG from plasma taken into cells. There is speculation in the literature regarding this for other cell types with putative CBG membrane receptors involved (32). The fact that corticosterone binding in rodent white adipose tissue mirrors plasma CBG levels (12) supports the idea that CBG may be sequestered in adipose tissue and/or taken up by preadipocytes/adipocytes. The facts that there is less CBG in the adipose tissue of obese rats than in that of lean rats, and that there are regional differences in CBG in adipose tissue, subcutaneous greater than visceral, strongly suggest that CBG is an important modulator of glucocorticoid activity in adipose tissue (12).

However, this does not explain why preadipocytes from a CBG-negative individual should respond differently than normal preadipocytes under identical in vitro conditions if preadipocytes do not express CBG. CBG influences the kinetic parameters of cortisol transport and clearance. In studies comparing subjects with normal or high CBG concentrations (using estrogen-containing oral contraceptives), increased CBG levels were associated with a reduced rate of cortisol clearance and an increased mass of circulating cortisol in a smaller volume of distribution (4). It may simply be that long-term growth in a CBG-deficient environment, in vivo, causes changes in preadipocyte growth and differentiation that are sustained in vitro for at least four passages. This is indirectly supported by the fact that there were no short-term differences in glucocorticoid responsiveness and no change in replication
rate with additional glucocorticoid or with the anti- 
glucocorticoid RU-486.

Alternatively, the lack of CBG during fetal develop-
ment may modulate glucocorticoid action, causing per-
manent changes in gene expression in preadipocytes.

CBG is present in a number of fetal tissues, with
temporal and spatial changes in CBG localization sug-
gest that CBG influences steroid hormone activity
in fetal tissues (33). Glucocorticoids are involved in
gene programming, with one example being that of

promoting effect of glucocorticoids on the differen-
tiation of human preadipocytes. We studied GR number, because the GR
is a significant regulator of glucocorticoid action
in many tissues and because glucocorticoids downregu-
late their own receptor. We also compared their 11β-
HSD activity, as local interconversion of cortisol and
cortisone is known to have significant effects on glu-
cocorticoid responsiveness at a tissue level, and again

glucocorticoids regulate (increase) type 1 11β-HSD ac-
tivity (6). There were no differences in either GR num-
er or characteristics or in 11β-HSD activity between

promoting effect of glucocorticoids on the differen-
tiation of CBG-negative preadipocytes may

promote adipose tissue deposition and explain the obe-
sity seen in individuals with genetic CBG deficiency.

Furthermore, these observations may be relevant to
obesity occurring with suppressed CBG concentrations
associated with hyperinsulinemia.

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