11β-Hydroxysteroid Dehydrogenase Type 1 Regulation by Intracellular Glucose 6-Phosphate Provides Evidence for a Novel Link between Glucose Metabolism and Hypothalamo-Pituitary-Adrenal Axis Function*

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Microsomal glucose-6-phosphatase-α (G6Pase-α) and glucose-6-phosphate transporter (G6PT) work together to increase blood glucose concentrations by performing the terminal step in both glycogenolysis and gluconeogenesis. Deficiency of the G6PT in liver gives rise to glycogen storage disease type 1b (GSD1b), whereas deficiency of G6Pase-α leads to GSD1a. G6Pase-α shares its substrate (glucose-6-phosphate; G6P) with hexose-6-phosphate-dehydrogenase (H6PDH), a microsomal enzyme that regenerates NADPH within the endoplasmic reticulum lumen, thereby conferring reductase activity upon 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). 11β-HSD1 interconverts hormonally active C11β-hydroxy steroids (cortisol in humans and corticosterone in rodents) to inactive C11-oxo steroids (cortisone and 11-dehydrocorticosterone, respectively). In vivo reductase activity predominates, generating active glucocorticoids. We hypothesized that substrate (G6P) availability to H6PDH in patients with GSD1b and GSD1a will decrease or increase 11β-HSD1 reductase activity, respectively. We investigated 11β-HSD1 activity in GSD1b and GSD1a mice and in two patients with GSD1b and five patients diagnosed with GSD1a. We confirmed our hypothesis by assessing 11β-HSD1 in vivo and in vitro, revealing a significant decrease in reductase activity in GSD1b animals and patients, whereas GSD1a patients showed a marked increase in activity. The cellular trafficking of G6P therefore directly regulates 11β-HSD1 reductase activity and provides a novel link between glucose metabolism and function of the hypothalamo-pituitary-adrenal axis.

A vital function of the liver is to provide glucose during fasting. This occurs through two principal pathways, gluconeogenesis and glycogenolysis, in each case yielding glucose 6-phosphate (G6P), which is then hydrolyzed by glucose-6-phosphatase (G6Pase), more recently described as G6Pase-α (1), to glucose (reviewed in Ref. 2). G6Pase-α is a transmembrane protein in the endoplasmic reticulum (ER) with the active enzyme site directed toward the ER lumen (2, 3). G6P must be translocated from cytosol to ER via a ubiquitously expressed G6P transporter (G6P7) before hydrolysis can occur. Patients and rodent models lacking components of the G6Pase-α, G6PT system emphasize the crucial role of this pathway in maintaining glucose homeostasis in the fasting state. Profound fasting hypoglycemia occurs in glycogen storage disease type 1; von Gierke’s disease (type 1a) caused by G6Pase-α deficiency; and type 1b caused by a G6P7 defect (2, 3). Recombinant mice with global deletion of G6Pase-α (4) and G6PT (5) have similar phenotypes with profound hypoglycemia.

G6Pase-α shares its substrate (G6P) with another enzyme within the ER, hexose-6-phosphate-dehydrogenase (H6PDH), that catalyzes the first two steps of an ER-specific “pentose phosphate pathway,” i.e. both G6P dehydrogenase and 6-phosphogluconolactonase reactions (Fig. 1) (6, 7). Our recent studies have indicated a pivotal link between H6PDH activity in the ER and the control of set point of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). 11β-HSD1 is an ER-bound enzyme (8) catalyzing the interconversion of inactive glucorticoids (cortisone in humans and 11-dehydrocorticosterone in rodents) and hormonally active glucorticoids (cortisol and corticosterone) (9). The reaction direction, which 11β-HSD1 catalyzes, is determined by the relative abundance of NADP+ and NADPH (10). In its native purified state, 11β-HSD1 acts as

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5 The abbreviations used are: G6P, glucose 6-phosphate; G6Pase-α, glucose-6-phosphatase-α; G6PT, glucose 6-phosphate transporter; H6PDH, hexose-6-phosphate dehydrogenase; GSD1a, glycogen storage disease type 1a; GSD1b, glycogen storage disease type 1b; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; 11-DHC, 11-dehydrocorticosterone; F, cortisol; E, cortisone; ER, endoplasmic reticulum; THF, tetrahydrocortisol; THE, tetrahydrocortisone; WT, wild type; AUC, area under the curve; Mops, 4-morpholinepropanesulfonic acid.


**EXPERIMENTAL PROCEDURES**

**Preparation of Mouse Liver Microsomes**—Mouse liver microsomes were prepared from recombinant male mice (n = 3) with global deletion of G6Pase-α, or separately, deletion of G6PT by differential centrifugation as described previously (19). The phenotype and background strain of these mice have been previously reported (4, 5). All animal experiments had the approval of the Institutional Animal Care and Use Committee and were performed according to procedures approved by that committee. Microsomal fractions were resuspended in a buffer containing 20 mM NaCl, 1 mM MgCl₂, 100 mM KCl, 20 mM Mops, pH 7.2, and were snap-frozen under liquid nitrogen. Microsomal protein concentration was determined using the Bio-Rad protein assay with bovine serum albumin as a standard as per the manufacturer’s instructions (Bio-Rad). The integrity of the microsomal membranes was assessed by using the mannos-6-phosphatase assay (20), which showed a latency greater than 95% in all preparations.

**11β-HSD1 Enzyme Activity Assays**—Micromes (30 μg) were preincubated at 37 °C for 20 min in Mops buffer with 1 mM of the H6PDH substrate G6P. 11β-HSD enzyme reactions were started by the addition of 200 nM 11-dehydrocorticosterone/500 nM corticosterone spiked with 20,000 cpm of tritiated 11-dehydrocorticosterone/corticosterone. All experiments were performed in triplicate. After incubation at 37 °C for 30 min, steroids were extracted with dichloromethane, separated by thin layer chromatography using a mobile phase of ethanol and chloroform (8:92), and quantified using a Bioscan 2000 image analyzer (Lablogic, Sheffield, UK) (19). The percentage of substrate metabolized in each experiment was 10% or less, ensuring that initial rates of metabolism were being measured.

**Immunoblotting**—SDS-PAGE was performed by the method of Laemmli (21) with 10 μg of mouse liver microsomal protein on 11% acrylamide minigels using a Bio-Rad Mini-PROTEAN II apparatus (Bio-Rad). Following electrophoresis, proteins were transferred to Immobilon-P membrane (Millipore Corp., Bedford, MA). Nonspecific protein binding was blocked by incubating membranes in 20% nonfat milk, 0.1% Tween 20 in phosphate-buffered saline at 25 °C for 1 h. Membranes were then incubated with an in-house raised polyclonal antibody to human H6PDH at a dilution of 1:1000 for 16 h at 4 °C. Following 3 × 10-min washes in phosphate-buffered saline, 0.1% Tween 20, membranes were incubated with secondary antibody (goat anti-rabbit IgG peroxidase-conjugate) at a dilution of 1:25,000 for 1.5 h at room temperature. Bound peroxidase-conjugated IgG was visualized using ECL detection kit (Amersham Biosciences, Buckinghamshire, UK) by exposing membranes to x-ray film (Kodak, France). Following stripping, membranes were reprobed with a polyclonal antibody to human 11β-HSD1 (1:1000) (22) in a similar method as above and incubated with goat anti-sheep IgG peroxidase secondary antibody.

**Analysis of Urine from GSD1a and GSD1b Mice**—Urine was collected on filter paper following bladder massage, and samples were pooled from three of each group: wild type (WT), GSD1b, and GSD1a. Three individual pooled samples were collected from WT and GSD1b, and a single pooled sample was analyzed from the GSD1a mice. The filter papers were cut up and eluted with water while vortexing and sonicating. Extracts were subjected to steroid analysis by gas chromatography/mass spectrometry as described previously (19, 23, 24). Multiple corticosterone metabolites were found in these analyses. Major metabolites were 6β-hydroxy and 20-dihydro metabolites of corticosterone. Prominent saturated components were hydroxylated (6α- or 11β-) derivatives of “tetrahydro”...
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(3α, 5α, or 3α,5β-) or “hexahydro” (additionally reduced at 20α- or 20β-) corticosterone or 11-DHC. The basic structure of each compound and establishment of it as a corticosterone or 11-DHC metabolite was easily determined by mass spectral fragmentation, although stereochemistry of the 5-hydrogen and the 20-hydroxyl could not be determined in the absence of authentic compounds. Quantitation was achieved by measuring the total-ion-current response for each peak and relating this to a known amount of internal standard, assuming an equal mass spectrometry response. For the purpose of this study, excretions of individual 11-oxo and 11β-hydroxy metabolites were separately summed, and the percentage of excretion of each group was calculated.

**Patient Studies**—Five patients with GSD1a were investigated (two males, three females; mean age ± S.D., 28 ± 1 years). All patients gave formal written consent for the study, which was approved by the local hospital ethics committee and carried out according to the recommendations of the declaration of Helsinki. All patients were diagnosed in early infancy by liver biopsy and G6Pase-α enzyme assay after presenting with severe hypoglycemia.

Urine steroid metabolite analysis was carried out on 24-h urine collections from five patients with GSD1a. In addition, we were able to obtain single spot urine samples from two children with GSD1b. All samples were analyzed by gas chromatography/mass spectrometry, as reported previously (23, 25), measuring free and conjugated cortisol metabolites. Urinary steroid metabolite ratios and total 24-h cortisol metabolite production rates are presented in Table 1. GSD1a patients have been compared with 36 health controls (mean age ± S.D., 28.4 ± 5.6 kg/m2). Individual values are presented for the two GSD1b patients, alongside the age-adjusted reference ranges (Table 1). The THF 5α-THF/THE, the cortols/cortolones, and the 11OH-androsterone + 11OH-ethiocholanolone/11-oxo-ethiocholanolone ratios represent acknowledged markers of global 11β-HSD1 activity, with a high ratio indicating increased 11β-HSD reductase activity.

At 2300 h, GSD1a subjects were given 1 mg of dexamethasone orally to suppress endogenous cortisol production. All subjects attended the Clinical Research Facility at 0900 h the following morning, and after baseline 0900 h measurements of cortisol and adrenocorticotropic hormone, a further 0.5 mg of dexamethasone and cortisone acetate (25 mg) were given orally. Serum cortisol and cortisone concentrations were then measured at 30-min intervals for 240 min. Serum cortisol was assayed using a chemiluminescent immunoassay (Bayer Advia 1200, Bayer Diagnostics, Newbury, UK) with interassay coefficients of variation of 10.2% at 76 nmol/liter, 7.7% at 528 nmol/liter, and 7.4% at 882 nmol/liter. Cortisone was assayed after extraction from serum followed by radioimmunoassay of the extract with 125I-cortisone and Sac-Cel® (IDS Ltd., Tyne and Weir, UK) second antibody separation. Results were compared with data from 34 age and body mass index-matched controls from our existing data base of normal controls.

**Statistical Analysis**—Statistical analysis of comparisons among groups was undertaken using the one-way analysis of variance with Tukey’s post hoc testing (for normal distribution) or Mann-Whitney rank sum test (for non-normal distribution). Area under the curve (AUC) analysis was performed using the trapezoidal method. All analyses were performed using the SigmaStat 3.1 software package (Systat Software, Inc. Point Richmond, CA).

**RESULTS**

**In Vitro Analysis of 11β-HSD1 Activity and Protein Expression in GSD1b and GSD1a Mice**—Liver microsomal preparations have been extensively used to assess the kinetics of all the enzyme systems analyzed in this study and are considered a representative model system of the ER (3, 13, 27, 28). 11β-HSD1 enzyme activity was assessed by examining the 11-reductase (11-DHC to corticosterone) and dehydrogenase (corticosterone to 11-DHC) activity in mouse liver microsomes from WT, GSD1b, and GSD1a animals.

In hepatic microsomes from the WT animals, reductase activity predominated (Fig. 2A) and was significantly higher than dehydrogenase activity (p < 0.001; Fig. 2A). In the microsomes of GSD1b animals, where there is no transporter protein present for G6P, reductase activity was significantly lower than that from WT animals (p < 0.001; Fig. 2A), and dehydrogenase activity was at a similar level to WT controls. Expression of H6PDH and 11β-HSD1 protein was similar in WT and GSD1b animals (Fig. 2B), indicating that changes in the levels of these proteins could not account for the differences in activity. In the GSD1a mice, both reductase and dehydrogenase activities were reduced when compared with WT animals. This unexpected finding was explained by a reduction in expression of 11β-HSD1 protein in these animals, despite no change in expression of H6PDH (Fig. 2B).
In Vivo Analysis of Corticosterone Metabolism in GSD1b and GSD1a Mice—In vivo assessment of 11β-HSD1 activity in mice was carried out using gas chromatography/mass spectrometry analysis of pooled urine collections (n = 3) from each group. WT mice were found to excrete almost exclusively 11β-hydroxy metabolites (92.3 ± 3.4%; mean ± S.E.) with only minor amounts of 11-oxo metabolites (Fig. 3A). The converse was true for the GSD1b mice, where the dominant steroids excreted in the urine were 11-oxo metabolites (92.3 ± 3.4% WT versus 60.2 ± 4.6%; GSD1b, p < 0.001; Fig. 3A). This pattern of metabolism was mirrored in vitro, where the ratio of reductase to dehydrogenase activity obtained from liver microsomal preparations of GSD1b mice was significantly lower than WT (5.21 ± 0.64 WT versus 1.13 ± 0.28 GSD1b, p < 0.01; Fig. 3B). Results from a single pooled urine sample from GSD1a mice indicated a similar level of 11β-hydroxy metabolites to WT (87.5%; Fig. 3A). This was consistent with the relative ratio of reductase:dehydrogenase activity seen in vitro, being comparable with that of WT animals (5.21 ± 0.64 WT versus 5.7 ± 0.7 GSD1a; Fig. 3B).

In Vivo Analysis of Cortisol Metabolism in GSD1a and GSD1b Patients. Urinary Cortisol Metabolite Excretion—In GSD1a patients, total cortisol production rate, as determined from the summation of the metabolite excretion in a 24-h urine collection (29), was significantly decreased (Table 1). In addition, the THF+5αTHF/THE, the cortols/cortolones, and the 11OH-androsterone+11OH-etiocholanolone/11-oxo-etiocholanolone ratios, all of which reflect global 11β-HSD1 activity, were significantly increased, in keeping with an increased cortisone to cortisol conversion. Absolute levels of THF and THE were significantly lower than in control subjects; however, there was little change in the relative activity of either 5α-reductase or 5β-reductase as measured by the THF/5αTHF and etiocholanolone/androsterone ratios (Fig. 4), although it is possible that there is a reduction in both 5α-reductase and 5β-reductase activity. Small numbers of cases of GSD1b patients precluded statistical analysis, but in contrast to patients with GSD1a, in two cases of GSD1b, we observed a relative decrease in 5α and/or increase in 5β-reductase activity (increased THF/5αTHF ratio). However, the etiocholanolone/androsterone ratio was within the reference range. Importantly, 11β-HSD1 activity, as measured by the THF+5αTHF/THE and the cortols/cortolones ratios, was decreased (Table 1). These were spot urines from overnight collections, and we were unable therefore to assess 24-h cortisol metabolite secretion rates.

In Vivo Analysis of Cortisol Metabolism in GSD1a Patients, Cortisol Regeneration from an Exogenous Cortisone Challenge—Hepatic 11β-HSD 1 activity, measured as the conversion of orally administered cortisone (25 mg) to cortisol after overnight dexamethasone suppression (30), was significantly elevated in the GSD1a group when compared with controls (n = 34) (mean cortisol AUC ± S.E. 248 ± 3 versus 75 ± 4 μmol/liter-min, p < 0.001; Fig. 5A), consistent with increased hepatic 11β-HSD1 activity and endorsing our observations from the urinary corticosteroid metabolite analysis. Serial cortisone measurements over the same time course were similar in both control and GSD1a patients (mean AUC 15 ± 3 versus 13 ± 5 μmol/liter-min, p = 0.4) (Fig. 5B), suggesting no impact upon renal 11β-HSD2 activity.

**TABLE 1**

**Urinary steroid metabolite analysis**

| GSD1a (n = 5) | Sex and age matched controls (n = 36) | GSD1b (n = 2) | Sex and age matched controls (n = 15) |
|---------------|--------------------------------------|---------------|--------------------------------------|
| Total cortisol metabolites (μg/24 h) | 5191 ± 1330** | 12502 ± 1106 | 0.28, 0.27 |
| THF (μg/24 h) | 742 ± 175* | 2015 ± 189 | 0.12, 0.11 |
| 5α-THF (μg/24 h) | 724 ± 166 | 1978 ± 251 | 0.18–0.42 |
| THE (μg/24 h) | 856 ± 278** | 4303 ± 429 | 0.62–1.71 |
| (THF+5α-THF)/THE | 1.94 ± 0.2*** | 0.96 ± 0.04 | 0.7–6.0 |
| Cortols/cortolones | 0.64 ± 0.1*** | 0.39 ± 0.02 | 0.32–1.35 |
| (11OH-andro+11OHetio)/11oxo-etio | 13.8 ± 1.0*** | 3.2 ± 0.2 | 0.37–1.08 |
| THF/5α-THF | 1.08 ± 0.2 | 1.43 ± 0.19 | 2.6, 4.47 |
| Etio/andro | 0.75 ± 0.14 | 0.94 ± 0.09 | 0.48, 0.59 |

Figure 3. In vivo and in vitro assessment of 11β-HSD1 activity in WT, GSD1b and GSD1a mice. A, comparison of the percentage of urinary metabolites in WT mice with GSD1b and GSD1a revealed that the percentage of 11β-hydroxy metabolites (black bars) were significantly decreased in the GSD1b group when compared with WT. Results from a single pooled sample of GSD1a urine showed a similar profile of metabolites as WT. B, the relative ratio of reductase:dehydrogenase activity from microsomal assays of WT, GSD1b, and GSD1a mice was significantly higher in both the WT and the GSD1a animals when compared with GSD1b. Values indicate mean ± S.E.; **, p < 0.01; *** p < 0.001; n = 3.
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The studies on humans lacking G6Pase-\(\alpha\) (GSD1a) show for the first time an impressive increase in 11\(\beta\)-HSD1 reductase activity in vivo, resulting in increased local generation of active glucocorticoid. In these patients, G6P availability to H6PDH is increased, resulting in enhanced NADPH generation for 11\(\beta\)-HSD1. Activity was inferred in humans through an increase in the urinary THF+5aTHF/THE, cortols/cortolones, and 11OH-androst-erone+11OH-etiocholanolone/11-oxo-etiocholanolone ratios and increased generation of circulating cortisol following an oral dose of cortisone acetate, with unchanged markers of 5a-reductase and 5\(\beta\)-reductase activity. We and others have extensively utilized the cortisol generation test post-cortisone as a marker of liver 11\(\beta\)-HSD1 activity; a reduced generation of cortisol has been observed in patients with obesity (30) and in subjects with apparent cortisone reductase deficiency (34). However, this is the first time an increase in cortisol concentrations following cortisone has been observed. The normal ratio of urinary C11\(\beta\)-hydroxy:C11-11-oxo metabolites of glucocorticoids in humans is \(1:1\), but in mice, this ratio is \(10:1\), reflecting a more efficient reductase enzyme (19). As a consequence, a further increase in the percentage of urinary 11\(\beta\)-hydroxy metabolites was not observed in mice lacking G6Pase-\(\alpha\). However, in hepatic microsomes from mice lacking G6Pase-\(\alpha\), absolute levels of both reductase and dehydrogenase activities were lower than WT controls. Since both reductase and dehydrogenase activities were reduced by the same extent, the ratio of these activities remained unchanged. This was explained by an overall reduction in expression of 11\(\beta\)-HSD1 in the liver of these animals which may represent a negative feedback mechanism whereby an attempt is made to increase G6P hydrolysis by limiting the requirement for G6P by H6PDH, although this hypothesis requires further investigation.

Conversely, when delivery of G6P to the ER is compromised, as seen in mice and humans lacking G6PPT (GSD1b), a significant impairment of 11\(\beta\)-HSD1 reductase activity was observed. It was not possible to undertake cortisol generation profiles following oral cortisone in two patients with GSD1b, but the urinary THF+5aTHF/THE ratio was reduced by 71\% when compared with controls. Similar changes were seen in mice, where the percentage of 11-oxo metabolites fell from 92.3 to 39.8\%. Our in vitro data indicated that this was a direct result of impaired reductase activity. These data compare favorably with those obtained from our H6PDH knockout mouse. In these animals, we also found that the set point of 11\(\beta\)-HSD1 activity switched from reductase to dehydrogenase with greater dehydrogenase activity evident from liver microsomal preparations.
and a higher percentage of 11-oxo metabolites present in the urine (19).

Glycogen storage disease type 1 is a complex liver disorder, and both GSD1a and GSD1b patients manifest the symptoms of failed G6P hydrolysis, characterized by growth retardation, hypoglycemia, hepatomegaly, nephromegaly, hyperlipidemia, hyperuricemia, and lactic academia (2). However, there are distinct phenotypic differences between GSD type 1a and 1b, which are not obviously related to G6P metabolism in glucocorticoid tissues, and local alterations in 11β-HSD1 activity may explain some of these differences. The pathogenesis of osteoporosis in GSD1a, which is not present in GSD1b, has been studied in some detail and demonstrates many similarities with glucocorticoid-induced bone loss (35, 36). Additionally, one unique feature of GSD1b that is not seen in GSD1a is neutropenia (37–40). G6PT is widely distributed, but G6Pase-one unique feature of GSD1b that is not seen in GSD1a is neutropenia (37–40). G6PT is widely distributed, but G6Pase-

<REFERENCES>
1. Shieh, J. J., Pan, C. J., Mansfield, B. C., and Chou, J. Y. (2003) J. Biol. Chem. 278, 47098–47103.
2. Chou, J. Y., Matern, D., Mansfield, B. C., and Chen, Y. T. (2002) Curr. Mol. Med. (Hilversum) 2, 121–143.
3. Van Schaftingen, E., and Gerin, I. (2002) Biochem. J. 362, 513–532.
4. Lei, K. J., Chen, H., Pan, C. I., Ward, J. M., Mosinger, B., Jr., Lee, E. J., Westphal, H., Mansfield, B. C., and Chou, J. Y. (1996) Nat. Genet. 13, 203–209.
5. Chen, L. Y., Shieh, J. J., Lin, B., Pan, C. J., Gao, J. L., Murphy, P. M., Roe, T. F., Moses, S., Ward, J. M., Lee, E. I., Westphal, H., Mansfield, B. C., and Chou, J. Y. (2003) Hum. Mol. Genet. 12, 2547–2558.
6. Hewitt, K. N., Walker, E. A., and Stewart, P. M. (2005) Endocrinology 146, 2539–2543.
7. Mason, P. J., Stevens, D., Diez, A., Knight, S. A., Scopes, D. A., and Vullarmy, T. I. (1999) Blood Cells Mol. Dis. 25, 30–37.
8. Ozols, J. (1995) J. Biol. Chem. 270, 2305–2312.
9. Tomlinson, J. W., Walker, E. A., Bujalska, I. J., Draper, N., Laverty, G. G., Cooper, M. S., Hewison, M., and Stewart, P. M. (2004) Endocr. Rev. 25, 831–866.
10. Lakshmi, V., and Monder, C. (1988) Endocrinology 123, 2390–2398.
11. Walker, E. A., Clark, A. M., Hewison, M., Ride, J. P., and Stewart, P. M. (2001) J. Biol. Chem. 276, 21343–21350.
12. Clarke, J. L., and Mason, P. J. (2003) Arch. Biochem. Biophys. 415, 229–234.
13. Banhegyi, G., Benedetti, A., Fulceri, R., and Senesi, S. (2004) J. Biol. Chem. 279, 27017–27021.
14. Bujalska, I. J., Draper, N., Michailidou, Z., Tomlinson, J. W., White, P. C., Chapman, K. E., Walker, E. A., and Stewart, P. M. (2005) J. Mol. Endocrinol. 34, 675–684.
15. Czegle, I., Piccirella, S., Senesi, S., Csaia, M., Mandl, J., Banhegyi, G., Fulceri, R., and Benedetti, A. (2006) Mol. Cell. Endocrinol. 248, 24–25.
16. Kotelevtsev, Y., Holmes, M. C., Burchell, A., Houston, P. M., Schmoll, D., Jamieson, P., Best, R., Brown, R., Edwards, C. R., Seckl, J. R., and Mullins, J. I. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 14924–14929.
17. Bujalska, I. J., Kumar, S., and Stewart, P. M. (1997) Lancet 349, 1210–1213.
18. Tomlinson, J. W., and Stewart, P. M. (2005) Nat. Clin. Pract. Endocrinol. Metab. 1, 92–99.
19. Laverty, G. G., Walker, E. A., Draper, N., Jeyasuria, P., Marcos, J., Shackleton, C. H., Parker, K. L., White, P. C., and Stewart, P. M. (2006) J. Biol. Chem. 281, 6546–6551.
20. Burchell, A., Hume, R., and Burchell, B. (1988) Clin. Chim. Acta 173, 183–191.
21. Laemmli, U. K. (1970) Nature 227, 680–685.
22. Ricketts, M. L., Verhaeg, I., Bujalska, I., Howie, A. J., Rainey, W. E., and Stewart, P. M. (1998) J. Clin. Endocrinol. Metab. 83, 1325–1335.
23. Shackleton, C. H. (1993) J. Steroid Biochem. Mol. Biol. 45, 127–140.
24. Shackleton, C. H., Lerdink, K., and Lawson, A. M. (1990) Mass Spectrometry in Biological Materials (Larsen, B. S., and McEwen, C. N., eds) pp. 297–377, CRC Press, Inc., New York.
25. Palermo, M., Shackleton, C. H., Mantero, F., and Stewart, P. M. (1996) Clin. Endocrinol. (Oxf) 45, 605–611.
26. Alberts, P., Nilsson, C., Selen, G., Engblom, L. O., Edling, N. H., Norling, S.,

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Klingstrom, G., Larsson, C., Forsgren, M., Ashkzari, M., Nilsson, C. E., Fiedler, M., Bergqvist, E., Ohman, B., Bjorkstrand, E., and Abrahmsen, L. B. (2003) Endocrinology 144, 4755–4762
27. Banhegyi, G., Marcolongo, P., Fulceri, R., Hinds, C., Burchell, A., and Benedetti, A. (1997) J. Biol. Chem. 272, 13584–13590
28. van de W. G., Lange, A., Newgard, C., Mechlin, M. C., Li, Y., and Berteloot, A. (2000) Eur. J. Biochem. 267, 1533–1549
29. Tomlinson, J. W., Moore, J. S., Clark, P. M., Holder, G., Shakespeare, L., and Stewart, P. M. (2004) J. Clin. Endocrinol. Metab. 89, 2711–2716
30. Stewart, P. M., Boulton, A., Kumar, S., Clark, P. M., and Shackleton, C. H. (1999) J. Clin. Endocrinol. Metab. 84, 1022–1027
31. Atanasov, A. G., Nashev, L. G., Schweizer, R. A., Frick, C., and Odermatt, A. (2004) FEBS Lett. 571, 129–133
32. McCormick, K. L., Wang, X., and Mick, G. J. (2006) J. Biol. Chem. 281, 341–347
33. Piccirella, S., Czegle, I., Lizak, B., Margittai, E., Senesi, S., Papp, E., Csala, M., Fulceri, R., Csermely, P., Mandl, J., Benedetti, A., and Banhegyi, G. (2006) J. Biol. Chem. 281, 4671–4677
34. Tomlinson, J. W., Draper, N., Mackie, J., Johnson, A. P., Holder, G., Wood, P., and Stewart, P. M. (2002) J. Clin. Endocrinol. Metab. 87, 57–62
35. Cabrera-Abreu, J., Crabtree, N. J., Elias, E., Fraser, W., Cramb, R., and Alger, S. (2004) J. Inherit. Metab. Dis. 27, 1–9
36. Rake, J. P., Visser, G., Huismans, D., Huitema, S., Van, d. V., Piers, D. A., and Smit, G. P. (2003) J. Inherit. Metab. Dis. 26, 371–384
37. Garty, B. Z., Douglas, S. D., and Danon, Y. L. (1996) Isr. J. Med. Sci. 32, 1276–1281
38. Gitzelmann, R., and Bosshard, N. U. (1993) Eur. J. Pediatr. 152, Suppl. 1, S33–S38
39. Beaudet, A. L., Anderson, D. C., Michels, V. V., Arion, W. J., and Lange, A. I. (1980) J. Pediatr. 97, 906–910
40. Visser, G., Rake, J. P., Labrune, P., Leonard, J. V., Moses, S., Ullrich, K., Wendel, U., Groenier, K. H., and Smit, G. P. (2002) Eur. J. Pediatr. 161, Suppl. 1, S83–S87
41. Paterson, J. M., Morton, N. M., Fievet, C., Kenyon, C. J., Holmes, M. C., Staels, B., Seck, I. R., and Mullins, J. I. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 7088–7093
42. Krebs, M., Kressak, M., Nowotny, P., Weghuber, D., Gruber, S., Mlynarik, V., Bischof, M., Stingl, H., Furnsinn, C., Waldhausl, W., and Roden, M. (2001) J. Clin. Endocrinol. Metab. 86, 2153–2160
43. Basu, R., Singh, R., Basu, A., Johnson, C. M., and Rizza, R. A. (2006) Diabetes 55, 667–674
44. Brandenberger, G., Follenius, M., and Hietter, B. (1982) J. Clin. Endocrinol. Metab. 54, 592–596
45. Follenius, M., Brandenberger, G., and Hietter, B. (1982) J. Clin. Endocrinol. Metab. 55, 757–761
46. Knoll, E., Muller, F. W., Ratge, D., Bauersfeld, W., and Wisser, H. (1984) J. Clin. Chem. Clin. Biochem. 22, 597–602
47. Ferguson, S. E., Pallikaros, Z., Cooke, B. A., and Michael, A. E. (1999) Mol. Cell. Endocrinol. 158, 37–44
48. Valsamakis, G., Anwar, A., Tomlinson, J. W., Shackleton, C. H., McTernan, P. G., Chetty, R., Wood, P. J., Banerjee, A. K., Holder, G., Barnett, A. H., Stewart, P. M., and Kumar, S. (2004) J. Clin. Endocrinol. Metab. 89, 4755–4761