Influence of Placental 11β-Hydroxysteroid Dehydrogenase (11β-HSD) Inhibition on Glucose Metabolism and 11β-HSD Regulation in Adult Offspring of Rats

Hirokazu Saegusa, Yuichi Nakagawa, Yan-Jun Liu, and Takehiko Ohzeki

Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts glucocorticoids to 11-keto-products and is believed to play an important role in protecting fetuses from higher maternal glucocorticoid levels. Recent reports have speculated that prenatal glucocorticoid exposure leads to fetal growth retardation and adult offspring hypertension and hyperglycemia. To investigate the effects of placental 11β-HSD2 inhibition on glucose metabolism and the 11β-HSD system in adult offspring, pregnant rats were treated with daily injections of carbenoxolone (CBX), an inhibitor of 11β-HSD. The offspring of the maternal CBX treatment group showed reduced birth weight (treated *v* control, 5.6 ± 0.5 *v* 6.4 ± 0.4 g, $P < .0001$). In adult offspring of the maternal CBX treatment group, plasma hemoglobin A_{1c} was significantly increased (7.3% ± 1.8% *v* 4.8% ± 0.3%, $P < .01$) and glucose intolerance was shown on the oral glucose tolerance test. The gene expression of hepatic 11β-HSD1 and renal 11β-HSD2 was decreased 87.6% ($P < .05$) and 52.3% ($P < .01$) in adult offspring of the maternal CBX treatment group, whereas renal 11β-HSD1 was not significantly altered. The change in 11β-HSD activity corresponded to the change in the gene expression. These results suggest that inhibition of placental 11β-HSD2 causes growth retardation, glucose intolerance, and partial suppression of the 11β-HSD system in the offspring.

PREVIOUS STUDIES have cloned and characterized two distinct isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD).[1,2] 11β-HSD1 is a low-affinity, NADP(H)-dependent dehydrogenase/oxoreductase,[3] whereas 11β-HSD2 is a high-affinity, NAD-dependent dehydrogenase.[4] These two enzymes are the product of separate genes and their mRNA expression exhibits tissue-specific patterns. 11β-HSD2 is highly expressed in placental syncytiotrophoblasts[5] and protects the fetus from higher (five to 10 times) maternal glucocorticoid levels by the rapid conversion of physiologically active forms (cortisol in humans and corticosterone in rats) into inert 11-keto-products (cortisone and 11-dehydrocorticosterone, respectively).[6]

Human epidemiological studies have shown that low birth weight is associated with an increased risk of hypertension,[7] ischemic heart disease,[8] and non–insulin-dependent diabetes mellitus (NIDDM)[9] in adult life. Other studies have demonstrated that excessive fetal exposure to maternal glucocorticoids leads to low birth weight and subsequent glucose intolerance in the offspring.[10] These data suggest that the intrauterine environment plays a crucial role in determining later glucose homeostasis. However, the mechanism responsible for the derangement in glucose metabolism has not been clarified. 11β-HSD is likely a key enzyme because it is present in high levels in the placenta and converts glucocorticoids into their physiologically inactive forms.

In this study, we evaluated the effects of placental 11β-HSD2 on the offspring, using the specific 11β-HSD antagonist carbenoxolone (CBX), to determine whether intrauterine steroid metabolism significantly influences metabolism in later life.

From the Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan.

Submitted February 23, 1999; accepted June 1, 1999.

Address reprint requests to Hirokazu Saegusa, MD, Department of Pediatrics, Hamamatsu University School of Medicine, 3600 Handa-Cho, Hamamatsu 431-3192, Japan.

0026-0495/99/4812-0021$10.00/0

MATERIALS AND METHODS

Animals and Treatment

Eight-week-old male Sprague-Dawley rats were obtained from Nippon SLC (Hamamatsu, Japan). The animals were housed under normal laboratory-controlled conditions (temperature 22° to 26°C and lights on between 7 AM and 7 PM), and rat chow and water were available ad libitum.

The rats were time-mated and then received daily injections of either CBX (12.5 mg/kg in 4% ethanol-saline 0.1 mL; Sigma, St Louis, MO) or vehicle alone (control) subcutaneously throughout pregnancy as previously reported.[11] At birth, neonatal rats were weighed and no further treatment was given.

Measurement of Blood Glucose, Plasma C-Peptide, and Hemoglobin A_{1c}

Male offspring underwent an oral glucose tolerance test at 1 year of age. The rats were fasted overnight, and glucose 2 g/kg was administered by gavage between 9 and 10 AM the following morning. Blood was collected in heparinized Eppendorf tubes by tail snipping at 0, 15, 30, 60, 90, and 120 minutes. Plasma was stored at −20°C.

The glucose concentration was measured by a glucose oxidase system (Boehringer, Mannheim, Germany). Plasma C-peptide was determined by double-antibody radioimmunoassay (RIA) using a rat C-peptide RIA kit (Linco Research, St Louis, MO). Hemoglobin A_{1c} was assayed by affinity chromatography (Glyc-Affin GHb; Isolab, Akron, OH).

Measurement of 11β-HSD Activity

11β-HSD activity was determined by measuring the rate of conversion of [^{3}H]corticosterone ([^{3}H]B) to [^{3}H]11-dehydrocorticosterone ([^{3}H]A) as previously reported.[12,13] Briefly, kidney or liver tissues (0.5 g) were homogenized in Krebs-Ringer buffer (KRB) solution at 4°C in a Dounce tissue grinder. The tissue homogenates were centrifuged, and the protein concentration of the supernatants was determined in a homogenate dilution by the method of Bradford (Bio-Rad Protein Assay Kit; Bio-Rad, Richmond, CA). The homogenate supernatants of kidney tissues were incubated in KRB containing 100 nmol/L [^{3}H]B (specific activity, 90 Ci/mmol; New England Nuclear, Boston, MA; 1 mCi) and 200 mmol/L NAD^+ as a cofactor for 10 minutes at 37°C in an agitated bath. The homogenate supernatants of liver tissues were incubated in KRB containing 2 μmol/L [^{3}H]B and 3.4 mmol/L NADP as a cofactor. The steroids were extracted with ethyl acetate and separated by thin-layer chromatography in a chloroform:ethanol (9:1) system. The

activity of 11β-HSD (renal 11β-HSD1 and 11β-HSD2 and hepatic 11β-HSD1) is expressed as the percentage conversion of [^{3}H]B to [^{3}H]A, which is calculated from the radioactivity of each fraction.

RNA Preparation and Northern Blot Analysis

Total RNA was extracted from the kidney or liver samples using the single-step guanidinium isothiocyanate acid-phenol chloroform extraction method as previously described.[14] For Northern blot analysis, aliquots of total RNA (20 mg) were subjected to electrophoresis, on 1.4% agarose gels and transferred to a nylon membrane (Hybond-N$^+$; Amersham International, Bucks, UK) by overnight capillary transfer, and the membrane was fixed under UV light. The membranes were prehybridized for 24 hours before adding the probes. To assist in the quantification of mRNA, rat 11β-HSD1 (1,265 base pairs [bp]) cDNA from Dr P.C. White[1] and rat 11β-HSD2 (1,864 bp) cDNA from Dr C.E. Gomez-Sanchez[2] were labeled with [^{32}P]dCTP (specific activity, 6,000 Ci/mmol; Amersham) using nick translation (Nick Translation System; BRL Life Technologies, Bethesda, MD). Autoradiographs were obtained by exposing the membranes to x-ray film with an intensifying screen at −70°C for up to 5 days. For densitometric measurements, autoradiographic signals were standardized to signals determined from 18S rRNA in each preparation to control for the amount of RNA loaded per lane.

Statistical Analysis

All data are expressed as the mean ± SD. Comparisons among groups were performed by the unpaired t test. Values are considered statistically significant at a P level less than .05.

RESULTS

CBX treatment of pregnant rats reduced the birth weight (CBX v control, 5.6 ± 0.5 v 6.4 ± 0.4 g, $P < .0001$) of offspring compared with the controls (Fig 1). There were no significant differences in gestation time, litter size, or viability among the groups.

At 1 year of age, the male offspring of rats treated with CBX during pregnancy showed significantly higher plasma hemoglobin A_{1c} (7.3% ± 1.8% v 4.8% ± 0.3%, $P < .01$) than the control group (Fig 2). After the oral glucose load, plasma glucose was significantly higher at 30, 60, 90, and 120 minutes in the CBX group (Fig 3A). Plasma C-peptide was also significantly higher at 15 and 30 minutes (Fig 3B). Body weight was similar among the groups at this time (data not shown).

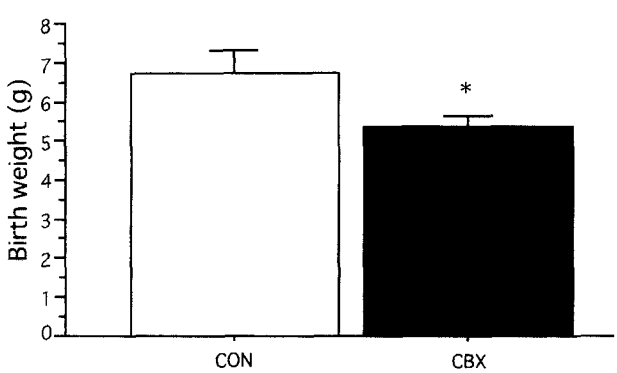

Fig 1. Birth weight of offspring of the maternal group treated during pregnancy with vehicle (CON, n = 34) or CBX (n = 32). *$P <$ .05 v CON.

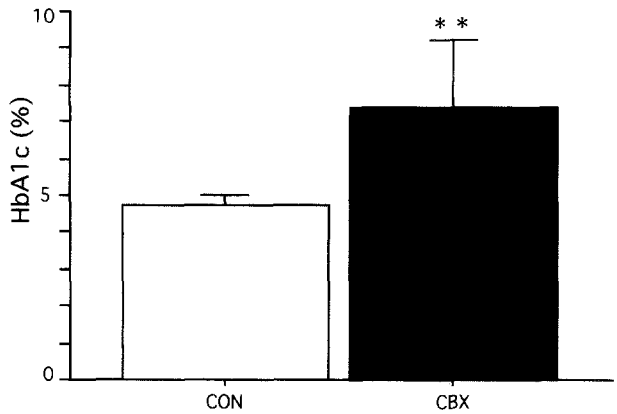

Fig 2. Plasma hemoglobin A_{1c} in offspring of the maternal group treated during pregnancy with vehicle (CON, n = 6) or CBX (n = 6). **$P <$ .01 v control.

Figure 4A to C shows the expression of 11β-HSD mRNA in the kidney or liver at 1 year of age. In the kidney, 11β-HSD1 expression was not significantly different in CBX offspring versus the controls, and 11β-HSD2 was significantly ($P < .01$) lower. In the liver, 11β-HSD1 expression was significantly ($P < .05$) reduced.

11β-HSD activity was determined by measuring the rate of conversion of [^{3}H]B to [^{3}H]A (Fig 5). CBX offspring showed significantly lower renal 11β-HSD2 activity ($P <$

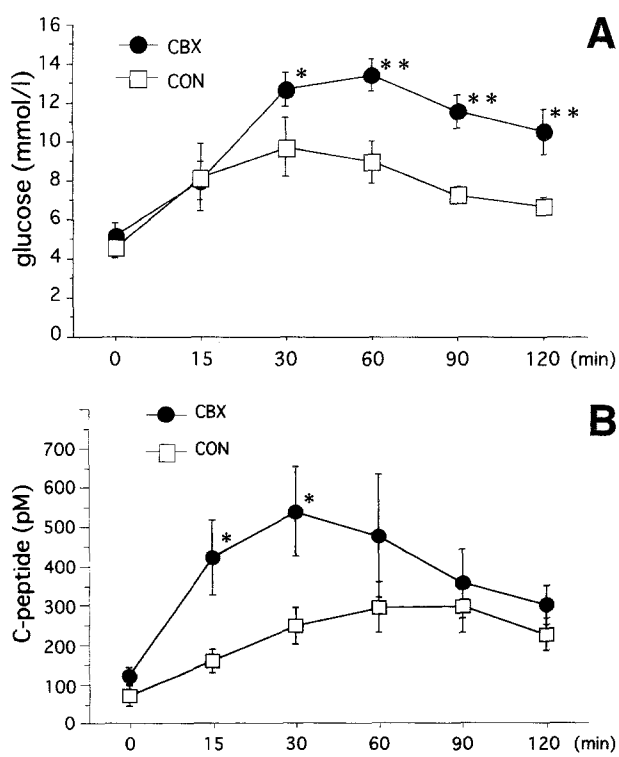

Fig 3. Plasma (A) glucose and (B) C-peptide on oral glucose tolerance test in offspring of maternal rats treated during pregnancy with vehicle (CON, n = 6) or CBX (n = 6). *$P <$ .05, **$P <$ .01 v control.

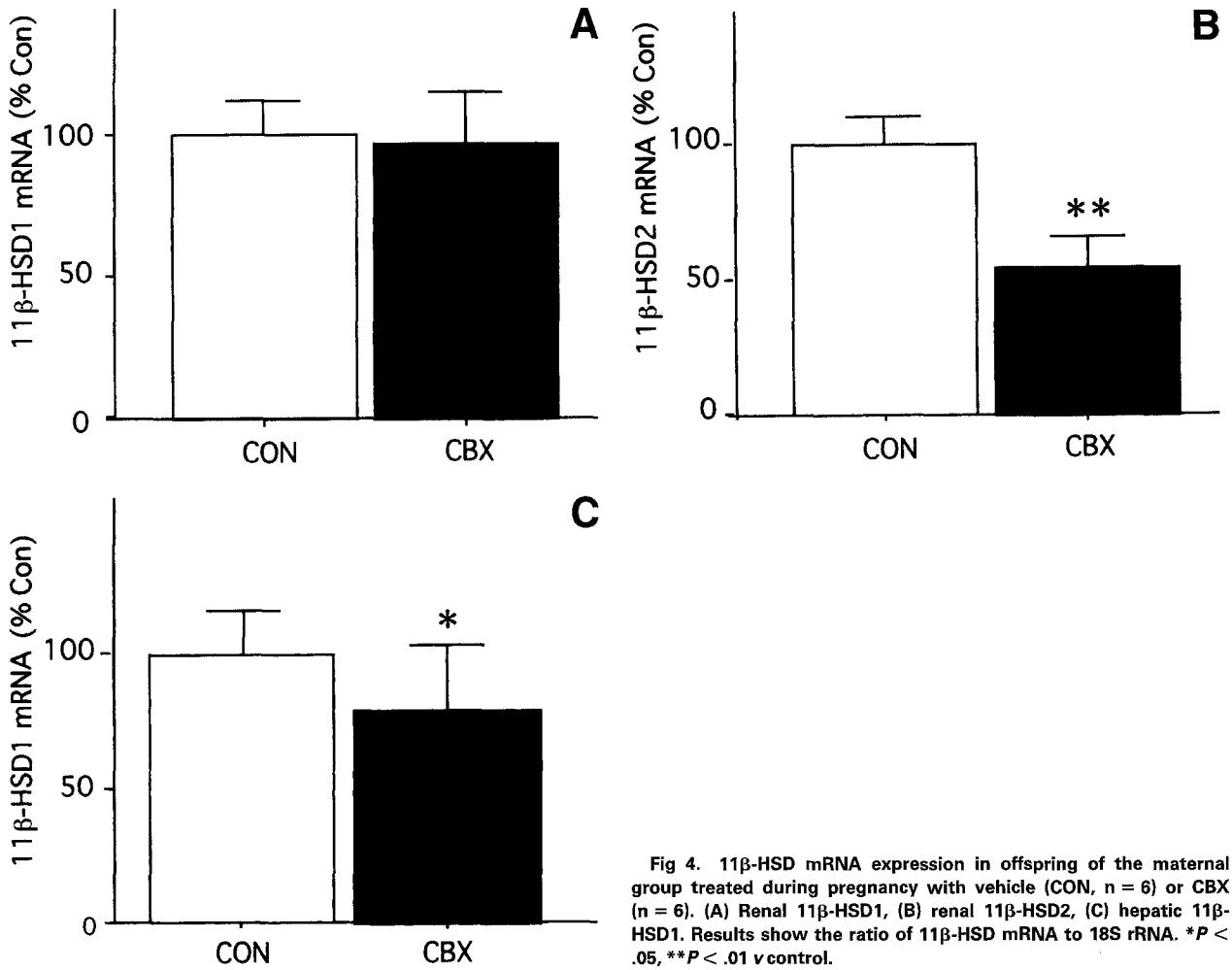

Fig 4. 11β-HSD mRNA expression in offspring of the maternal group treated during pregnancy with vehicle (CON, n = 6) or CBX (n = 6). (A) Renal 11β-HSD1, (B) renal 11β-HSD2, (C) hepatic 11β-HSD1. Results show the ratio of 11β-HSD mRNA to 18S rRNA. *$P <$ .05, **$P <$ .01 *v* control.

.01) and hepatic 11β-HSD1 activity ($P < .05$) than the control group. Renal 11β-HSD1 activity was not significantly different among the groups. These results are similar to the findings for 11β-HSD mRNA expression.

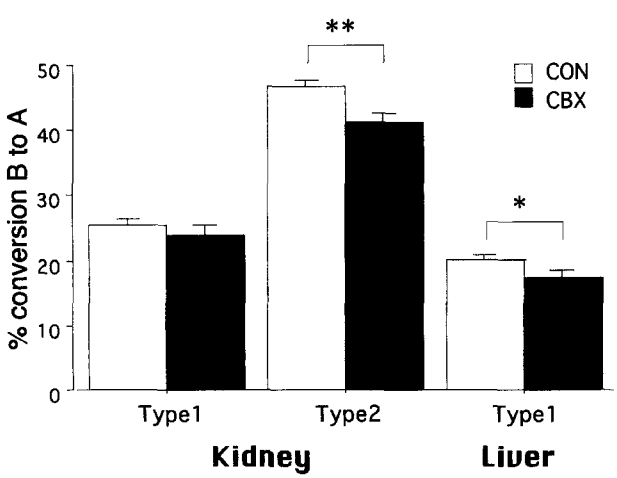

Fig 5. 11β-HSD activity in offspring of the maternal group treated during pregnancy with vehicle (CON, n = 6) or CBX (n = 6). Results are expressed as the percentage conversion of [^{3}H]B to [^{3}H]A. *$P <$ .05, **$P <$ .01 *v* control.

DISCUSSION

Glucocorticoids have well-characterized hypertensive and hyperglycemic effects.[15] It was recently proposed that prenatal glucocorticoid exposure programs blood pressure[11] and glucose tolerance[10] in the later life of the offspring. Placental 11β-HSD2, which is highly expressed in syncytiotrophoblasts,[5] is considered to protect the fetus from the higher glucocorticoid levels of the mother by rapid conversion of the physiologically active forms (cortisol in humans and corticosterone in rats) into inert 11-keto-products (cortisone and 11-dehydrocorticosterone, respectively).[6]

We administered CBX to pregnant rats to inhibit placental 11β-HSD2. In a recent study, maternal CBX treatment was demonstrated to reduce placental 11β-HSD2 activity to 63.5% of the control level.[10] In this study, the treatment caused a reduction of birth weight, glucose intolerance, and partial suppression of 11β-HSD. These results may not be directly applicable to humans, because there is a possibility that steroid metabolism is distinct in rats versus humans. However, positive correlations between birth weight and placental 11β-HSD2 activity were reported in rats[16] and humans.[17] Moreover, some patients with apparent mineralocorticoid excess, which is caused by low 11β-HSD2 activity due to a mutation in the 11β-HSD2 gene, were shown to have a low birth weight.[18] It

has been considered that birth weight is related to placental 11β-HSD2, because a decrease of placental 11β-HSD2 activity impaired fetal growth due to a failure to inactivate maternally derived glucocorticoid.[19]

At 1 year of age in rats, almost the same as 60 years of age in humans, we investigated glucose metabolism and 11β-HSD alternation in adult offspring. Hemoglobin A_{1c}, which is known as one of the most sensitive and specific indicators for diabetes mellitus in humans and animals, was significantly higher in the offspring of the CBX treatment group. This finding shows that the offspring of the CBX group had persistently higher glucose concentrations, although the fasting glucose level was not different between the two groups. The glucose load caused significant elevations in blood glucose and increased C-peptide. The findings indicate that the insulin resistance results in NIDDM.

Although Lindsay et al[10] reported hyperglycemia in the offspring of CBX-treated mothers, the alternation of 11β-HSD has not been reported. Nyirenda et al[20] reported that dexamethasone treatment in rats during late pregnancy caused offspring glucose intolerance and increased glucocorticoid receptor mRNA expression, but did not change hepatic 11β-HSD1 mRNA expression. Our data indicate that maternal CBX treatment simultaneously decreased hepatic 11β-HSD1 and renal 11β-HSD2 regulation in adult offspring. We propose two possibilities. One is the different effect on the fetus between CBX and dexamethasone. Maternal CBX treatment might program 11β-HSD regulation in the offspring. The other is the suppression of hepatic 11β-HSD1 by elevated serum insulin observed as an inhibitory effect on 11β-HSD1 in humans[21] and rats.[22] Furthermore, we previously demonstrated that streptozotocin-induced insulin-dependent diabetic rats had increased renal 11β-HSD1 and decreased renal 11β-HSD2 activity and gene expression.[23] Hyperglycemia probably induces a reduction in the intracellular NAD^+:NADH ratio[24] and results in a decrease of renal 11β-HSD2 activity. Concerning renal 11β-HSD1, the inhibitory effects of insulin and the acceleratory effects of glucose may counteract each other. It is unclear whether the suppression of 11β-HSD resulted from impairment of glucose metabolism or the glucose intolerance resulted from the programed decrease of 11β-HSD. Incidentally, there has been no mention as to whether patients with apparent mineralocorticoid excess developed hyperglycemia or diabetes in later life. It is probably considered that the patients have a low enzyme activity of 11β-HSD2, but not 11β-HSD1, which is known to have more important roles in glucose metabolism.

It has been speculated that prenatal exposure to excessive levels of glucocorticoid programs the glucose metabolism, leading to permanent hyperglycemia and insulin resistance.[10] The programing may act via many pathways. Firstly, prenatal stress or glucocorticoid exposure permanently program an increased activity of the hypothalamic-pituitary-adrenal axis, producing glucocorticoid hypersecretion.[25] This mechanism is largely mediated by a reduced number of glucocorticoid receptors in the hippocampus, which is an important locus of negative-feedback control.[26] As demonstrated in previous investigations in human fetuses with a birth weight below the 10th percentile for gestational age, cord levels of venous cortisol, corticotropin, and corticotropin-releasing factor were increased and dehydroepiandrosterone sulfate was decreased.[27,28] Secondly, glucocorticoid affects hepatic enzymes involved in glucose metabolism, most notably phosphoenolpyruvate carboxykinase (PEPCK), a key enzyme in gluconeogenesis and an important target gene in NIDDM.[29] As recent experiments show, PEPCK expression is increased by maternal dexamethasone treatment in pregnancy.[20] Overexpression of PEPCK in a rat hepatoma cell line or in transgenic mice impaired the suppression of gluconeogenesis.[30] Thirdly, glucocorticoid stimulates the insulin-like growth factor (IGF) system. Hepatic production of IGF binding protein-1 (IGFBP-1) is markedly induced by maternal dexamethasone treatment.[31] IGFBP-1 may sequester free IGF, decreasing its bioavailability, and block the insulin-like actions of free IGFs.[32] Transgenic mice overexpressing IGFBP-1 show a low birth weight and adult hyperglycemia.[33]

In the present study, we have demonstrated that the inhibition of placental 11β-HSD is associated with low birth weight, glucose intolerance, and partial suppression of 11β-HSD in the offspring of the maternal CBX treatment group. It has been suggested that an alternation of intrauterine glucocorticoid metabolism causes not only fetal growth retardation but also a derangement in metabolism, including glucose tolerance and 11β-HSD regulation, in later life.

REFERENCES

1. Agarwal AK, Monder C, Eckstein B, et al: Cloning and expression of rat cDNA encoding corticosteroid 11β-dehydrogenase. J Biol Chem 264:18939-18943, 1989

2. Zhou M-YI, Gomez-Sanchez EP, Cox DL, Cosby D, Gomez-Sanchez CE: Cloning, expression, and tissue distribution of the rat nicotinamide adenine dinucleotide-dependent 11β-hydroxysteroid dehydrogenase. Endocrinology 136:3729-3734, 1995

3. Tannin GM, Agarwal AK, Monder C, et al: The human gene for 11β-hydroxysteroid dehydrogenase structure, tissue distribution, and chromosomal localization. J Biol Chem 266:16653-16658, 1991

4. Albiston AL, Obeyesekere VR, Smith RE, et al: Cloning and tissue distribution of the human 11β-hydroxysteroid dehydrogenase type 2 enzyme. Mol Cell Endocrinol 105:R11-R17, 1994

5. Brown RW, Chapman KE, Kotelevtsev Y, et al: Cloning and production of antisera to human placental 11β-hydroxysteroid dehydrogenase type 2. Biochem J 313:1007-1017, 1996

6. Murphy BEP, Clark SJ, Donald IR, et al: Conversion of maternal cortisol to cortisone during placental transfer to the human fetus. Am J Obstet Gynecol 118:538-541, 1974

7. Barker DJ, Osmond C, Golding J, et al: Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease. BMJ 298:564-567, 1989

8. Barker DJ, Gluckman PD, Godfrey KM, et al: Fetal nutrition and cardiovascular disease in adult life. Lancet 41:938-941, 1993

9. Hales CN, Barker DJP, Clark PMS: Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 303:1019-1022, 1991

10. Lindsay RS, Lindsay RM, Waddell BJ, et al: Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: Studies with the 11β-hydroxysteroid dehydrogenase inhibitor carbenoxolone. Diabetologia 39:1299-1305, 1996

11. Lindsay RS, Lindsay RM, Edwards CRW, et al: Inhibition of 11β-hydroxysteroid dehydrogenase in pregnant rats and the program-

ming of blood pressure in the offspring. Hypertension 27:1200-1204, 1996

12. Stewart PM, Whorwood CB, Valentino R, et al: 11β-Hydroxysteroid dehydrogenase activity and gene expression in the hypertensive Bianchi-Milan rat. J Hypertens 11:349-354, 1993

13. Liu YJ, Nakagawa Y, Toya K, et al: Sex-specific effects of growth hormone on hepatic 11β-hydroxysteroid dehydrogenase activity and gene expression in hypothyroid rats. Life Sci 61:325-334, 1997

14. Kubota A, Nakagawa Y, Igarashi Y: Studies of gene expression in liver of insulin like growth factor (IGF)-1, IGFBP-3 and growth hormone (GH) receptor/GH binding protein in rats treated neonatally with monosodium glutamine. Horm Metab Res 26:497-503, 1994

15. Ross E, Marshall-Jones P, Friedman M: Cushing's syndrome: Diagnostic criteria. Q J Med 35:149-193, 1966

16. Benediktsson R, Lindsay RS, Noble J, et al: Glucocorticoid exposure in utero: New model for adult hypertension. Lancet 341:339-341, 1993

17. Stewart PM, Rogerson FM, Mason JI: Type 2 11β-hydroxysteroid dehydrogenase messenger ribonucleic acid and activity in human placenta and fetal membranes: Its relationship to birth weight and putative role in fetal adrenal steroidogenesis. J Clin Endocrinol Metab 80:885-890, 1995

18. Kitanaka S, Tanae A, Hibi I: Apparent mineralocorticoid excess due to 11β-hydroxysteroid dehydrogenase deficiency: A possible cause of intrauterine growth retardation. Clin Endocrinol (Oxf) 44:353-359, 1996

19. Shams M, Kilby MD, Somerset DA, et al: 11β-Hydroxysteroid dehydrogenase type 2 in human pregnancy and reduced expression in intrauterine growth restriction. Hum Reprod 13:799-804, 1998

20. Nyirenda MJ, Lindsay RS, Kenyon CJ, et al: Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. J Clin Invest 101:2174-2181, 1998

21. Hammami MM, Siiteri PK: Regulation of 11β-hydroxysteroid dehydrogenase activity in human skin fibroblasts: Enzyme modulation of glucocorticoid action. J Clin Endocrinol Metab 73:326-334, 1991

22. Liu YJ, Nakagawa Y, Nasuda K, et al: Effect of growth hormone, insulin and dexamethasone on 11β-hydroxysteroid dehydrogenase activity on a primary culture of rat hepatocytes. Life Sci 59:227-234, 1996

23. Liu YJ, Nakagawa Y, Ohzeki T: Gene expression of 11β-hydroxysteroid dehydrogenase type 1 and type 2 in the kidneys of insulin-dependent diabetic rats. Hypertension 31:885-889, 1998

24. Ido Y, Kilo C, Williamson JR: Cytosolic NADH/NAD, free radicals, and vascular dysfunction in early diabetes mellitus. Diabetologia 40:S115-S117, 1997 (suppl)

25. Walker BR, Williams BC, Edwards CR: Regulation of 11β-hydroxysteroid dehydrogenase activity by the hypothalamic-pituitary-adrenal axis in the rat. J Endocrinol 141:467-472, 1994

26. Low SC, Moisan MP, Noble JM, et al: Glucocorticoids regulate hippocampal 11β-hydroxysteroid dehydrogenase activity and gene expression in vivo in the rat. J Neuroendocrinol 6:285-290, 1994

27. Economides DL, Nicolaides KH, Linton EA, et al: Plasma cortisol and adrenocorticotropin in appropriate and small for gestational age fetuses. Fetal Ther 3:158-164, 1988

28. Goland LS, Jozak S, Warren WB, et al: Elevated levels of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses. J Clin Endocrinol Metab 77:1174-1179, 1993

29. Friedman JE, Yun JS, Patel YM, et al: Glucocorticoids regulate the induction of phosphoenolpyruvate carboxykinase (GTP) gene expression during diabetes. J Biol Chem 268:12952-12957, 1993

30. Valera A, Pujol A, Pelegrin M, et al: Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non–insulin-dependent diabetes. Proc Natl Acad Sci USA 91:9151-9154, 1994

31. Price WA, Stiles AD, Moats-Staats BM, et al: Gene expression of insulin like growth factors (IGFs), the type 1 IGF receptor, and IGF binding protein in dexamethasone-induced fetal growth retardation. Endocrinology 130:1424-1432, 1995

32. Ooi GT: Insulin like growth factor binding proteins: More than just 1, 2, 3. Mol Cell Endocrinol 71:C39-C43, 1990

33. Rajkuhmar K, Barron D, Lewitt MS, et al: Growth retardation and hyperglycemia in insulin like growth factor binding protein-1 transgenic mice. Endocrinology 136:4029-4034, 1995