Treatment with an SSRI antidepressant restores hippocampo-hypothalamic corticosteroid feedback and reverses insulin resistance in low-birth-weight rats

Esben S. Buhl,¹ ² ⁵ ⁶ Thomas Korgaard Jensen,¹ ² Niels Jessen,¹ ² Betina Elving,⁴ Christian S. Buhl,¹ ² Steen B. Kristiansen,³ Rasmus Pold,¹ ² Lasse Solskov,¹ ² Ole Schmitz,¹ Gregers Wegener,⁴ Sten Lund,² and Kitt Falck Petersen¹ ⁵ ⁶

¹Department of Pharmacology, Aarhus University; ²Department of Medicine (Endocrinology and Diabetes) and Medical Research Laboratory and ³Medical Department B (Cardiology), Aarhus University Hospital, Aarhus; ⁴Centre for Psychiatric Research, Aarhus University Hospital, Risskov, Denmark; and ⁵Department of Internal Medicine, Section of Endocrinology, Yale University School of Medicine, New Haven, Connecticut

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Buhl ES, Jensen TK, Jessen N, Elving B, Buhl CS, Kristiansen SB, Pold R, Solskov L, Schmitz O, Wegener G, Lund S, Petersen KF. Treatment with an SSRI antidepressant restores hippocampo-hypothalamic corticosteroid feedback and reverses insulin resistance in low-birth-weight rats. Am J Physiol Endocrinol Metab 298: E920–E929, 2010. First published January 26, 2010; doi:10.1152/ajpendo.00606.2009—Low birth weight (LBW) rats exposed to fetal glucocorticoid exposure (ESC) could downregulate HPA axis activity and restore insulin sensitivity in LBW rats. After 4–5 wk of treatment, ESC-exposed LBW (SSRI-LBW) and saline-treated control and LBW rats (Cx and LBW) underwent an oral glucose tolerance test or a hyperinsulinemic euglycemic clamp to assess whole body insulin sensitivity. Hepatic phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression and red skeletal muscle PKB Ser⁴⁷³ phosphorylation were used to assess tissue-specific insulin sensitivity. mRNA expression of the hypothalamic mineralocorticoid receptor was fivefold upregulated in LBW (P < 0.05 vs. Cx), accompanied by increased corticosterone release during restraint stress and total 24-h urinary excretion (P < 0.05 vs. Cx), while body insulin resistance (P < 0.001 vs. Cx), and impaired insulin suppression of hepatic PEPCK mRNA expression (P < 0.05 vs. Cx). Additionally, there was a tendency for reduced red muscle PKB Ser⁴⁷³ phosphorylation. The ESC treatment normalized corticosterone secretion (P < 0.05 vs. LBW), whole body insulin sensitivity (P < 0.01) as well as postprandial suppression of hepatic mRNA PEPCK expression (P < 0.05), and red muscle PKB Ser⁴⁷³ phosphorylation (P < 0.01 vs. LBW). We conclude that these data suggest that the insulin resistance and chronic HPA axis hyperactivity in LBW rats can be reversed by treatment with an ESC, which downregulates HPA axis activity, lowers glucocorticoid exposure, and restores insulin sensitivity in LBW rats.

selective serotonin reuptake inhibitors

STRESS MAY BE INVOLVED IN THE DEVELOPMENT OF major Western lifestyle disorders such as cardiovascular disease and the metabolic syndrome (53, 59, 61). Particularly in humans born with low birth weight (LBW) (e.g., birth weight <2,500 g), stress-related psychiatric illness and metabolic disturbances seem to coexist, and the prevalence of conditions associated with psychological stress, such as melancholic depression, is increased in subjects born with LBW (35). In addition, these individuals also have a higher prevalence of type 2 diabetes (23, 57, 80) that is potentially due to early development of insulin resistance. Hence, the LBW condition can be considered as both a predepressive and a prediabetic state. However, the exact mechanisms responsible for these changes and whether they are associated are still debated.

Recently, impairments in hippocampal structure and function have been proposed to account for some of the phenotypic characteristics of LBW (21, 40, 63). Hippocampus regulates the overall circadian tonus of the hypothalamic-pituitary-adrenal (HPA) axis hyperactivity. We examined whether treatment with a selective serotonin reuptake inhibitor [eslicotolapram (ESC)] could downregulate HPA axis activity and restore insulin sensitivity in LBW rats. After 4–5 wk of treatment, ESC-exposed LBW (SSRI-LBW) and saline-treated control and LBW rats (Cx and LBW) underwent an oral glucose tolerance test or a hyperinsulinemic euglycemic clamp to assess whole body insulin sensitivity. Hepatic phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression and red skeletal muscle PKB Ser⁴⁷³ phosphorylation were used to assess tissue-specific insulin sensitivity. mRNA expression of the hypothalamic mineralocorticoid receptor was fivefold upregulated in LBW (P < 0.05 vs. Cx), accompanied by increased corticosterone release during restraint stress and total 24-h urinary excretion (P < 0.05 vs. Cx), while body insulin resistance (P < 0.001 vs. Cx), and impaired insulin suppression of hepatic PEPCK mRNA expression (P < 0.05 vs. Cx). Additionally, there was a tendency for reduced red muscle PKB Ser⁴⁷³ phosphorylation. The ESC treatment normalized corticosterone secretion (P < 0.05 vs. LBW), whole body insulin sensitivity (P < 0.01) as well as postprandial suppression of hepatic mRNA PEPCK expression (P < 0.05), and red muscle PKB Ser⁴⁷³ phosphorylation (P < 0.01 vs. LBW). We conclude that these data suggest that the insulin resistance and chronic HPA axis hyperactivity in LBW rats can be reversed by treatment with an ESC, which downregulates HPA axis activity, lowers glucocorticoid exposure, and restores insulin sensitivity in LBW rats.

selective serotonin reuptake inhibitors

* These authors contributed equally to this article.

Address for reprint requests and other correspondence: E. S. Buhl, Dept. of Pharmacology, The Bartholin Bldg., Wilhelm Meyers Allé, Bldg. 1240, Aarhus University, DK-8000 Aarhus C, Denmark (e-mail: Esb@farm.au.dk).
development of both type 2 diabetes and depression in LBW subjects.

The antidepressant selective serotonin reuptake inhibitors (SSRIs) are capable of downregulating HPA axis activity in rats and humans (32, 34, 52), and some studies further suggest that SSRIs can improve glucose metabolism in depressed nondiabetic patients as well as in depressed patients displaying obesity and/or type 2 diabetes (43, 69, 74). Since depression, obesity, and type 2 diabetes are generally associated with abnormal regulation of glucocorticoid metabolism (3, 6, 67, 75), we therefore hypothesized that downregulation of HPA axis activity using a SSRI compound would lower glucocorticoid levels and thereby improve glucose metabolism and insulin action in LBW.

In adult, 40-day-old, LBW rats subjected to prenatal dexamethasone exposure, we aimed to study the effects of SSRI [escitalopram (ESC)] treatment on the hippocampo-hypothalamic corticosteroid feedback system, pituitary ACTH secretion and adrenal corticosterone production as well as whole body insulin action, hepatic mRNA expression of gluconeogenic enzymes, and insulin sensitivity of fat and skeletal muscle tissue. The present rat model displays the combination of insulin resistance and HPA axis hyperactivity (17), features similar to those of the adult LBW man.

**MATERIALS AND METHODS**

**Animals.** Experiments were approved by the Danish Animal Experiments Inspectorate and complied with The European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes. Pregnant Sprague-Dawley dams (Taconic) were housed in a light- (light from 6 AM to 6 PM) and temperature-controlled (22°C) environment and had free access to food and water. Pregnant dams (n = 39) were treated daily from day 14 to day 21 of gestation, with 0.15 mg/kg body wt dexamethasone sc in a 4% ethanol-saline (0.9% NaCl) solution or with 4% ethanol-saline (0.9% NaCl). At birth, pups (n = 273) regardless of the prenatal treatment (e.g., saline or dexamethasone) were transferred to nontreated, healthy, and lactating foster mothers (n = 39) in comparably sized groups of 6–8 pups/litter. Pups were weaned at the age of 3 wk, and only male offspring were included for further studies (n = 133).

For 4–5 wk, 40-day-old male rats were injected intraperitoneally twice a day with either ESC-saline solution (diluted oral droplets of Cipralex obtained at the pharmacy) at a concentration of 1.4 mg/ml, pH = 4.95 (10 mg/kg body wt), or saline (pH = 4.95). A washout period of 16–18 h after the last injection was allowed prior to all experiments. Rats were euthanized during isoflurane-anesthesia or pentobarbital (clamped animals), and all tissues were snap-frozen and stored at −20°C until further analysis. The first group of saline-treated control (Cx) and LBW rats (n = 37) underwent the oral glucose tolerance test (OGTT), the second group (n = 30) underwent the hyperinsulinemic euglycemic clamp, the third group was used for basal muscle and liver tissue analysis (n = 23), and in the fourth group (n = 27) brain tissue was collected. In a pilot study, the effects of ESC on OGTT and corticosterone secretion were assessed in Cx rats (n = 16).

**Restraint stress test.** The restraint stress test was performed as described previously (17) at 9 AM. Tail blood samples were drawn into heparinized capillary tubes at t = 0, 15, 30, 45, 60, 75, and 90 min after restraint was initiated. Blood was transferred to NaF-coated tubes, centrifuged at 2,000 rpm, and stored at −20°C until further analysis.

**Twenty-four-hour urine sampling.** Rats were housed for 24 h from 10 AM in metabolic cages, and urine was collected as described previously (17). Debris was removed by centrifugation and urine kept at −20°C until further analysis.

OGTT. After a 16-h overnight fast, 2.5 mg glucose/kg body wt was administered by gavage. Tail blood samples were obtained and placed into NaF-coated tubes at 0, 15, 30, 60, and 120 min after the glucose challenge. Plasma was stored at −20°C until further analysis.

**Insulin sensitivity index.** The insulin sensitivity index (ISI) was calculated according to Matsuda and DeFronzo (44): FG, fasting plasma glucose concentration (mg/dl); FI, fasting plasma insulin concentration (mU/l). Mean OGTT glucose and mean OGTT insulin are the average concentrations of blood glucose and plasma insulin, respectively, during the OGTT. The ISI represents the composite whole body insulin sensitivity, reflecting both hepatic and peripheral tissue insulin sensitivity.

**Metabolites and hormones.** Plasma and urine concentrations of insulin and corticosterone were measured by a rat insulin ELISA kit (DRG Instruments) and a rat corticosterone radioimmunoassay (Amersham), respectively. Blood glucose concentrations were measured using a One-Touch Ultra blood glucose analyzer.

**Hepatic mRNA expressions.** Hepatic mRNA was isolated by use of a RNA easy column kit (Qiagen, Valencia, CA). Complementary DNA was synthesized, and mRNA levels were assessed by quantitative real-time PCR (qPCR; TaqMan) using an ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA). The primer sets used were glucose-6-phosphatase (G-6-Pase): forward primer AGG GTA AAA GAA AAG AGT GT G and reverse primer GTA GAC ATG GCT TGC ATA TGT T; phosphoenolpyruvate carboxykinase (PEPCK): forward primer CAG GAA GTG AGG AAG TTT GTG G and reverse primer ATG ACA CCC TCC TCC TGC AT; and GAPDH: forward primer TCACCACCATGGAAGGC and reverse primer GCTAAG-CAGTGGTGTTGGCA. Pilot studies revealed that GAPDH was not differentially regulated in these rats, and threshold cycle values of for G-6-Pase and PEPCK were normalized relative to GAPDH (Supplemental Table S1; Supplemental Material for this article is available at the AJP-Endocrinology and Metabolism web site).

**Real-time qPCR of hippocampus and hypothalamus.** The rats were decapitated, and hypothalamus and hippocampus were dissected and frozen on dry ice powder. Total RNA was isolated using the ABI PRISM 6100 Nucleic Acid Prepetition (Applied Biosystems) and reverse transcribed using random primers and Superscript III Reverse Transcriptranscriptase (Invitrogen, Carlsbad, CA), as described previously (22). The real-time qPCR reactions were carried out by using the MX3000P (Stratagene, La Jolla, CA) and SYBR Green. The gene expression of glucocorticoid receptor (GR), mineralocorticoid receptor (MR), 11β-hydroxysteroid dehydrogenase-1 (HSD-1), corticotropin-releasing hormone (CRH) receptor-1 and -2 (CRH-R1 and CRH-R2, respectively), CRH-binding protein (CRH-BP), the apoptosis genes Bax and Bcl-2, and eight different reference genes [18S subunit ribosomal RNA (18S), β-actin, cyclophilin A, Gapdh, hydroxymethylbilane synthase, hypoxanthine guanine phosphoribosyl transferase 1, ribosomal protein L13A, and tyrosine 3-monoxygenase-tryptophan 5-monooxygenase activation protein-c] was investigated. The reference genes were selected as described by Bonefeld et al. (13). Stability comparison of the expression of the reference genes was conducted with the Normfinder software (http://www.mdl.dk) (Supplemental Table S1) (2).

**Hyperinsulinemic euglycemic clamp.** One week prior to the euglycemic hyperinsulinemic clamp experiment, indwelling catheters were placed as described previously (61) into the jugular vein for infusions and into the left carotid artery for blood collections, filled with a polyvinylpyrrolidone-heparine solution, and closed.

After 12-h overnight fast, catheters were flushed with saline, and the 150-min hyperinsulinemic clamp was initiated with a primed continuous insulin infusion in awake and unrestrained rats (primed: 200 mU/kg body wt; continuous: 4 mU·kg⁻¹·min⁻¹) (Actrapid; Novo Nordisk, Bagsvaerd, Denmark). Blood glucose levels were kept at ~100 mg/dl by a variable infusion of 20% d-glucose. Plasma glucose concentrations were measured using a Glucose Analyzer II (Beckman Instruments, Fullerton, CA).
To estimate rates of insulin-stimulated glucose transport activity in red (RG) and white gastrocnemius (WG) muscle tissue and epididymidal fat pads, a single dose of 20 μCi 2-[1-14C]deoxyglucose was administered at t = 120 min. Plasma-specific activity of 2-[1-14C]deoxyglucose was measured at t = 121, 123, 125, 130, 135, 140, and 150 min, and the concentrations of plasma glucose were used to estimate glucose uptake activity in tissues, as described previously (17).

*Clamp tissue glucose uptake assay.* The tissues were homogenized 1:10 (w/vol) in demineralized H2O and placed in a heat block at 100°C for 10 min. Samples were cooled to room temperature and centrifuged for 5 min. In supernatants, the total activity of 2-[1-14C]deoxyglucose as well as the phosphorylated (intracellular) and unphosphorylated (extracellular) fractions of 2-[1-14C]deoxyglucose were counted following separation by use of anion exchange chromatography columns (cat. no. 731-6211; Bio-Rad Laboratories, Hercules, CA).

*Skeletal muscle tissue glycogen levels.* Muscles were heated for 120 min at 99°C in 2.0 N HCl. Afterward, samples were neutralized with 2.0 N NaOH and centrifuged at 10,000 g for 1 min. Sample glucose levels were assayed using hexokinase reagent (CIMA Scientific, De Soto, TX), as described previously (12).

**RG PKB protein expression and Ser273 phosphorylation.** Muscle tissue was homogenized as described previously (79). Phospho-Ser273 total PKB antibodies were obtained from Cell Signaling Technology (Beverly, MA). Western blots were developed using enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ) and quantified using the UVP BioImaging System (UVP, Upland, CA).

**Total crude membrane GLUT4 contents.** Crude membranes were prepared from ~30 mg of RG and WG muscles, and aliquots of protein were resolved as described previously by use of a polyclonal anti-COOH-terminal peptide GLUT4 antibody (16). Protein bands were visualized by chemiluminescence (Pierce Super Signal) and subsequently quantified by use of the UVP BioImaging System.

**Statistical analysis.** Data are given as means ± SE. Data were analyzed with the one-way ANOVA test and Newman-Keuls post hoc test. P < 0.05 was considered statistically significant.

**RESULTS**

**Body weights, fat content, and food consumption.** Birth weights of the LBW rats were 17% less than the control rats (Cx: 6.40 ± 0.05 g, n = 72, vs. LBW: 5.32 ± 0.04 g, n = 66; P < 0.001). In a subgroup of random animals, no differences were seen with regard to the weight of epididymidal fat pads (Cx: 3.25 ± 0.18, n = 8; LBW: 2.71 ± 0.12 g, n = 8; SSRI-LBW: 2.89 ± 0.24, n = 7; P = 0.1279). At the end of the treatment period, body weights were the same for all groups (Cx: 352.9 ± 5.4 g, n = 44; LBW: 356.1 ± 10.4 g, n = 38; SSRI-LBW: 346.7 ± 9.8 g, n = 35; P = 0.7474). Furthermore, in a subgroup of rats (data not shown), food consumption and body weight development during treatment and rates of spontaneous activity, blood pressure, and body temperature as measured by telemetry at the end of the treatment period did not differ between any of the experimental groups.

**Restraint stress test.** Basal concentration of plasma corticosterone (the main glucocorticoid in the rat) did not differ (Fig. 1). Upon restraint stress in the Cx group, plasma corticosterone concentrations increased approximately fourfold at a peak to 30 min followed by a gradual decline throughout the test. A similar initial increase was observed in the LBW group; however, plasma cortisol concentrations remained approximately fourfold elevated until the end of the test. The area under the curve from 60 to 90 min (AUC60–90) was 56% larger in the LBW group than in the Cx group (Cx: 12,470 ± 1,270 ng/min vs. LBW: 19,440 ± 1,240 ng/min, P < 0.05; Table 1), suggesting impaired adrenal negative feedback inhibition in the LBW rats. The ESC treatment completely reversed this impairment of the adrenal stress response such that both the time course for plasma corticosterone concentrations and the AUC60–90 were similar to the Cx control group (SSRI-LBW: 11,180 ± 1,030 ng/min).

**Twenty-four-hour urine corticosterone excretion.** Urinary corticosterone excretion was increased by 38% in the LBW group compared with the Cx rats (Cx: 12,470 ± 1,270 ng/min vs. LBW: 19,440 ± 1,240 ng/min, P < 0.05; Table 1), suggesting defects in the basal circadian rhythm of corticosterone production in this group. This increase was completely reversed in the SSRI-LBW group (SSRI-LBW: 11,180 ± 1,030 ng/min).

**Plasma ACTH levels.** Although not significant, plasma ACTH levels at both 9 AM and 9 PM tended to be higher in the LBW group than in the Cx group (Table 1). During the day, plasma ACTH levels decreased moderately, by ~12 to 14% in the Cx and LBW groups, although this was significant only for the Cx group (P < 0.05 vs. 9 AM) and not for the LBW group (P = 0.286 vs. 9 AM), suggesting a possible defect in the diurnal control of ACTH secretion.

In contrast, plasma ACTH concentrations in the SSRI-LBW group were similar to the Cx rats at both 9 AM and 9 PM and decreased by ~24% during the day (P < 0.01 vs. 9 AM; Table 1).

**The hypothalamic and hippocampic glucocorticoid feedback axis.** The mRNA expression of HSD-1 and the GR in the hippocampus and hypothalamus was similar in all three groups (Table 1). In contrast, hypothalamic mRNA expression of the MR was approximately fivefold upregulated in LBW compared with Cx (Cx: 100 ± 20.4% vs. LBW: 501.7 ± 160.4%, P < 0.05). This was completely normalized in the SSRI-LBW group (205.8 ± 60.0, P < 0.05 vs. LBW), reflecting a down-regulation of the MR as a result of the ESC treatment.

There was a similar strong, albeit not statistically significant, tendency for upregulation of mRNA expression of the MR in the hippocampus of the LBW group (Cx: 100.0 ± 10.0% vs. LBW: 123.2 ± 13.3%, P = 0.072). However, in the SSRI-LBW group the hippocampic MR mRNA expression was normal and even tended to be lower than in the Cx group (SSRI-LBW: 85.5 ± 8.9% of Cx, n = 7), suggesting a potential modulation of the glucocorticoid feedback system.
within the LBW hippocampus as a result of ESC treatment. Furthermore, the hypothalamic and hippocampal mRNA expression patterns of CRH-R1, CRH-R2, and CRH-BP did not display any differences (data not shown). Similarly, the hippocampal mRNA expression patterns of the apoptosis genes Bcl-2 and Bax were also unaffected by the phenotype and treatments (data not shown). Similarly, the hippocampal corticosteroid feedback expression patterns of CRH-R1, CRH-R2, and CRH-BP did not display any differences (data not shown). Similarly, the hippocampal corticosteroid feedback expression patterns of CRH-R1, CRH-R2, and CRH-BP did not display any differences (data not shown).

**OGTT.** The LBW group had elevated blood glucose concentrations at 120 min of the OGTT ($P < 0.01$, LBW: 7.26 ± 0.04 mmol/l, $n = 11$, vs. Cx: 6.44 ± 0.03 mmol/l, $n = 13$; Fig. 2A), but SSRI treatment reversed this elevation ($P < 0.05$, SSRI-LBW: 6.12 ± 0.02 mmol/l, $n = 13$, vs. LBW; Fig. 2A). Whereas glucose AUC and fasting blood glucose were similar in the LBW and Cx groups, the OGTT glucose AUC was reduced by 15% in the SSRI-LBW group compared with the LBW group ($P < 0.01$ vs. LBW and $P < 0.05$ vs. Cx; Table 2).

Both fasting and postprandial plasma insulin concentration were two- to threefold increased in the LBW rats ($P < 0.01$ vs. Cx; Table 2) but were reduced significantly in ESC-exposed LBW rats ($P < 0.01$ vs. LBW; Table 2).

Accordingly, the whole body ISI in the LBW group was ~50% lower compared with the Cx group ($P < 0.01$; Table 2), which is consistent with whole body insulin resistance in the LBW group. However, ESC treatment almost normalized the ISI of the SSRI-LBW group ($P < 0.05$ vs. LBW; Table 2), reflecting restoration of whole body insulin sensitivity.

**Hepatic PEPCK and G-6-Pase mRNA expressions.** Basal hepatic PEPCK mRNA levels were similar in all three experimental groups and were suppressed by ~60% in the Cx group 90 min after administration of the oral glucose load ($P < 0.01$ vs. basal; Fig. 2B). In contrast, LBW rats suppressed PEPCK mRNA expression by only ~30% after oral glucose administration ($P < 0.05$ vs. Cx), strongly suggesting hepatic insulin resistance and inability for glucose-induced suppression of hepatic gluconeogenesis in the LBW liver.

### Table 1. Characterization of hippocampus and HPA axis

<table>
<thead>
<tr>
<th></th>
<th>Cx ($n = 5–13$)</th>
<th>LBW ($n = 8–14$)</th>
<th>SSRI-LBW ($n = 8–16$)</th>
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<tr>
<td><strong>Adrenal corticosterone release</strong></td>
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<tr>
<td>RST AUC 60-90 min, ng/min</td>
<td>12.470 ± 1.270</td>
<td>19.440 ± 1.240*</td>
<td>11.180 ± 1.030</td>
</tr>
<tr>
<td>24-h Urinary corticosterone excretion, mmol·24 h⁻¹·kg body wt⁻¹</td>
<td>10.1 ± 0.6</td>
<td>13.9 ± 1.2†</td>
<td>8.1 ± 0.5</td>
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<tr>
<td><strong>Pituitary ACTH release</strong></td>
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<tr>
<td>Plasma ACTH concentrations, pg/ml</td>
<td>15.96 ± 0.65</td>
<td>18.13 ± 1.40</td>
<td>16.87 ± 1.01</td>
</tr>
<tr>
<td>9 AM</td>
<td>13.18 ± 0.37‡</td>
<td>15.53 ± 1.42</td>
<td>12.89 ± 0.37‡</td>
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<tr>
<td><strong>Hypothalamic corticosteroid feedback</strong></td>
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<tr>
<td>HSD-1 mRNA expression, %Cx</td>
<td>100.0 ± 20.4</td>
<td>82.5 ± 16.1</td>
<td>73.6 ± 13.7</td>
</tr>
<tr>
<td>MR mRNA expression, %Cx</td>
<td>100.0 ± 21.4</td>
<td>501.7 ± 160.4‡</td>
<td>205.8 ± 60.0</td>
</tr>
<tr>
<td>GR mRNA expression, %Cx</td>
<td>100.0 ± 15.8</td>
<td>123.9 ± 15.8</td>
<td>121.6 ± 46.5</td>
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<tr>
<td><strong>Hippocampal corticosteroid feedback</strong></td>
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<tr>
<td>HSD-1 mRNA expression, %Cx</td>
<td>100.0 ± 15.5</td>
<td>122.2 ± 25.3</td>
<td>67.5 ± 8.6</td>
</tr>
<tr>
<td>MR mRNA expression, %Cx</td>
<td>100.0 ± 10.0</td>
<td>123.2 ± 13.3#</td>
<td>85.5 ± 8.9</td>
</tr>
<tr>
<td>GR mRNA expression, %Cx</td>
<td>100.0 ± 14.1</td>
<td>122.5 ± 11.1</td>
<td>88.1 ± 10.9</td>
</tr>
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</table>

Data are means ± SE. HPA, hypothalamic-pituitary-adrenal; RST, restraint stress test; AUC, area under the curve; Cx, saline-treated control; SSRI, selective serotonin reuptake inhibitor; LBW, low birth weight; HSD-1, 11β-hydroxysteroid dehydrogenase-1; MR, mineralocorticoid receptor; GR, glucocorticoid receptor. $P$ values for comparisons reflect the results for the post hoc analysis that has been carried out only when the 1-way of analysis of variance (ANOVA) test results in a $P$ value $<0.05$. (Fig. 1). Post hoc test $P$ values: *$P < 0.01$ vs. Cx and vs. SSRI-LBW; †$P < 0.05$ vs. Cx and $P < 0.01$ vs. SSRI-LBW; ‡$P < 0.05$ vs. 9 AM; §$P < 0.05$ vs. Cx; #$P = 0.0725$ vs. Cx.
Red muscle tissue PKB Ser\textsuperscript{473} phosphorylation. Basal PKB Ser\textsuperscript{473} phosphorylation in RG muscle was similar in all three groups (Table 3). In the Cx group, insulin stimulation was accompanied by a 135% increase in RG PKB Ser\textsuperscript{473} phosphorylation (\(P < 0.05\) vs. basal; Table 3). This insulin stimulation was decreased by 60% in the LBW rats, and although this did not reach statistical significance, it suggests impaired intracellular insulin signaling in RG muscle in the LBW rats. Insulin caused an 240% increase in RG PKB Ser\textsuperscript{473} phosphorylation in the SSRI-LBW rats (\(P < 0.01\) vs. LBW; Table 3). Together, these observations strongly suggest that ESC administration leads to a tissue-specific improvement in insulin signaling in RG muscle of LBW rats.

Pilot study assessing the effects of ESC in Cx rats (SSRI-Cx). The SSRI-Cx rats (\(n = 14–16\)) had similar food intake and weight gain during treatment (data not shown). After treatment, body weights (355.4 ± 6.3 g), fasting plasma concentrations of glucose (4.58 ± 0.12 mmol/l), fasting plasma concentrations of insulin (11.85 ± 1.62 mU/l), and glucose levels during OGTT (AUC:

<table>
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<th>Table 2. OGTT</th>
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<tr>
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<td>Fasting glucose concentrations, mmol/l</td>
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<tr>
<td>Fasting insulin concentrations, mU/l</td>
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<tr>
<td>OGTT total AUC\textsubscript{glucose}, mmol\cdot l\textsuperscript{-1} \cdot min\textsuperscript{-1}</td>
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<tr>
<td>OGTT total AUC\textsubscript{insulin}, mU\cdot l\textsuperscript{-1} \cdot insulin\textsuperscript{-1} \cdot min\textsuperscript{-1}</td>
</tr>
<tr>
<td>ISI (expressed as %Cx)</td>
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</table>

Data are means ± SE. OGTT, oral glucose tolerance test; ISI, insulin sensitivity index. \(P\) values for comparisons reflect the results for the post hoc analysis that has been carried out only when the 1-way ANOVA test results in a \(P\) value < 0.05. Post hoc test \(P\) values: *\(P < 0.01\) vs. Cx and \(P < 0.05\) vs. SSRI-LBW; †\(P < 0.05\) vs. Cx and \(P < 0.01\) vs. LBW; ‡\(P < 0.01\) vs. Cx and SSRI-LBW; §\(P < 0.01\) vs. Cx and \(P < 0.05\) vs. SSRI-LBW.
compared with LBW.

indicating that ESC affects HPA axis differently in Cx rats to 90 min (16,648
but tended to increase restraint stress corticosterone AUC from 60
§
P/H11021
axis. In all of the groups we found a diurnal decrease in ACTH
fecting disturbances in the hypothalamic control of the HPA
itivity can be attributed to defects at both hypothalamic and
increased ACTH secretion (17, 64). The HPA axis hyperactiv-
tivity, pituitary hyperplasia of the ACTH-producing cells, and
shown to be insulin resistant and to have HPA axis hyperac-
fection during both stress and basal conditions, respectively. In
early postnatal development of impaired glucose metabolism and hypertension (17, 47, 68).

though we did not measure CRH expression in our
CRH expression within the periventricular nucleus of the
Welberg et al. (78) have previously demonstrated increased
EFFECTS OF SSRI TREATMENT IN LOW-BIRTH-WEIGHT RATS

Table 3. Muscle glucose metabolism

<table>
<thead>
<tr>
<th></th>
<th>Cx (n = 5–14)</th>
<th>LBW (n = 8–9)</th>
<th>SSRI-LBW (n = 6–8)</th>
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<tbody>
<tr>
<td>Glycogen and GLUT4 protein content in RG and WG gastrocnemius muscle tissue</td>
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<tr>
<td>Basal glycogen WG, μmol/g muscle wet wt</td>
<td>38.8 ± 1.3</td>
<td>40.5 ± 1.7</td>
<td>42.9 ± 1.8</td>
</tr>
<tr>
<td>Basal glycogen RG, μmol/g muscle wet wt</td>
<td>31.4 ± 2.2*</td>
<td>33.8 ± 1.7*</td>
<td>32.4 ± 2.3*</td>
</tr>
<tr>
<td>Total GLUT4 content WG, arbitrary units</td>
<td>9.89 ± 3.36</td>
<td>10.32 ± 0.90</td>
<td>9.97 ± 0.58</td>
</tr>
<tr>
<td>Total GLUT4 content RG, arbitrary units</td>
<td>13.93 ± 0.71*</td>
<td>16.38 ± 1.92*</td>
<td>13.22 ± 0.98*</td>
</tr>
<tr>
<td>PKB Ser473 phosphorylation in RG muscle tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal phosphorylation, arbitrary units</td>
<td>29,568 ± 3,239</td>
<td>31,691 ± 4,901</td>
<td>22,459 ± 4,429</td>
</tr>
<tr>
<td>Clamp phosphorylation, arbitrary units</td>
<td>69,596 ± 10,600†</td>
<td>56,729 ± 7,641†</td>
<td>76,127 ± 8,287††</td>
</tr>
<tr>
<td>Insulin-stimulated increase from basal, %</td>
<td>135 ± 4</td>
<td>79 ± 24</td>
<td>239 ± 43§</td>
</tr>
</tbody>
</table>

869 ± 23 mmol glucose/min; 2-h concentrations: 6.34 ± 0.28
mmol/l) were not different from Cx. Surprisingly, however,
OGTT insulin concentrations were elevated significantly in
SSRI-Cx animals (AUC: 3,032 ± 74 μU.l⁻¹.min, P < 0.01 vs. Cx),
and the ISI was lower than in Cx (ISI: 73.6 ± 6.3% of Cx,
P < 0.05 vs. Cx), indicating that ESC causes insulin resistance in
SSRI-Cx rats. Furthermore, ESC did not change 24-h urinary
corticosterone excretion (11.0 ± 2.8 mmol·24 h⁻¹·kg⁻¹; n = 5)
but tended to increase restraint stress corticosterone AUC from 60
to 90 min (16,648 ± 1,745 ng/min, n = 10, P = 0.1201 vs. Cx),
indicating that ESC affects HPA axis differently in Cx rats
compared with LBW.

DATA

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Discussion

In clinical studies of preeclampsia and intrauterine growth
retardation, conditions known to cause LBW in humans, umbilical
cord blood samples have revealed elevated glucocorticoid
concentrations (25, 26). Furthermore, various animal
models for LBW such as maternal malnutrition (11), maternal
stress (41, 42), and late gestation uterine ligation (8) have all
been shown to significantly increase fetal glucocorticoid levels.
Hence, enhanced fetal glucocorticoid stimulation seems to be
the common denominator for both human and animal LBW
conditions, and therefore, as in our previous studies, we opted
to study a model of LBW where fetal growth retardation was
induced directly by prenatal treatment with dexamethasone,
a glucocorticoid analog capable of freely passing the blood-
placenta barrier, and to induce a robust glucocorticoid stimula-
tion of the fetuses (17). Similar to LBW humans, this model
is characterized by early postnatal development of impaired
glucose metabolism and hypertension (17, 47, 68).

In this model of LBW, we studied the effects of ESC

treatment on insulin sensitivity and HPA axis activity in a rat
model of LBW (17, 64). This model has previously been
shown to be insulin resistant and to have HPA axis hyperac-
tivity, pituitary hyperplasia of the ACTH-producing cells, and
increased ACTH secretion (17, 64). The HPA axis hyperactiv-
ity can be attributed to defects at both hypothalamic and
hippocampal levels (64) causing augmented corticosterone re-
lease during both stress and basal conditions, respectively. In
accord with our previous studies, the LBW rats presented
prolonged adrenal corticosterone release during restraint,
reflecting disturbances in the hypothalamic control of the HPA
axis. In all of the groups we found a diurnal decrease in ACTH
levels, which tended to be diminished in the LBW groups
compared with the Cx and the ESC-treated LBW groups. In
addition, 24-h urine excretion of corticosterone was increased
significantly and accompanied by disturbances of the circadian
rhythm of ACTH secretion, indicating concurrent defects in the
tonic hippocampal coordination of pituitary-adrenal activity.

O’Regan et al. (49) found the circadian oscillations of
ACTH to be at peak in the evening. This difference between
their data and ours is unclear, but their levels of ACTH were
∼15- to 20-fold higher than in other studies, including ours
(70), which could be due to differences in the timing of the
blood sampling, differences in assays used, and/or stress during
the blood sampling procedure.

In the hypothalamus and hippocampus, corticosteroids exert
a direct inhibitory feedback by binding to the MR and GR,
whereas HSD-1 assists this process by converting inactive
deoxycorticosteroid with low affinity for these receptors to
active corticosteroid. Because the MR exhibits an
15- to 20-fold higher than in other studies, including ours
ACTH to be at peak in the evening. This difference between
their data and ours is unclear, but their levels of ACTH were

we performed qPCR experiments, and normalization with different reference
genes may affect the obtained data differently (13). Also, circadian oscillations and different experimental settings easily
influence the measurements and emphasize the difficulties in
direct comparisons of such data between studies.

Welberg et al. (78) have previously demonstrated increased
CRH expression within the periventricular nucleus of the
hypothalamus of a similar model of dexamethasone-induced
LBW. Although we did not measure CRH expression in our

6–8)
study, we measured mRNA expression levels of the CRH receptors and the CRH-BP. CRH-R1 and CRH-R2 are widely expressed in the brain, and in states of hyperactive central CRH systems a concomitant dysregulation of the magnitude and duration of CRH receptor signaling is thought to contribute to an enhanced CRH neurotransmission (30). Further disturbances in CRH-BP, also a modulator of CRH activity, are also likely to play a role. In the rat hypothalamus and hippocampus, CRH-R1, CRH-R2, and CRH-BP are differentially expressed throughout different nuclei. In particular, hippocampal and hypothalamic R1 and R2 receptors take part in initiation (CRH-RH1) and recovery/maintenance of the stress response (CRH-RH2) (19, 24) as well as the regulation of serotonergic neurotransmission (CRH-R1) (53). However, here we found no differences in the hypothalamic or hippocampal mRNA expression pattern of these proteins in either of the experimental groups. Since chronic stress has been proposed to cause atrophy of the hippocampus, we also measured hippocampal mRNA expression patterns of two proteins involved in cellular apoptosis (i.e., Bax and Bcl-2), but we did not find any changes. Structural hippocampal change following a restraint prenatal stress paradigm has been observed, but only in females (62). Whether this is relevant in the context of the present work awaits future studies.

In LBW rats, the mRNA expressions of MR, which is quantitatively the most important protein for mediating negative corticosteroid feedback in the brain, were higher in hypothalamus and tended to be higher in the hippocampus compared with Cx. Despite these changes, LBW rats exhibited increased corticosteroid secretion and signs of disturbed ACTH secretion. Together, these alterations may suggest a compensatory upregulation of the MR due to impairments within the hippocampo-hypothalamic corticosteroid feedback regulation. These abnormalities were completely restored in the ESC-treated LBW animals, which displayed normal expressions of the MR in hypothalamus and hippocampus and had a normal 24-h urinary corticosterone excretion as well as a normal diurnal control (9 AM to 9 PM) of pituitary ACTH release. These findings strongly support the hypothesis that ESC treatment restores the HPA axis control in the LBW phenotype.

The mRNA expression data do not necessarily predict protein levels or protein function, due to possible posttranslational modifications or phosphorylations/nitrolylations of the protein. In addition, there are several limitations with regard to the quantitative PCR on dissected hippocampal and hypothalamic tissues. Although the method is superior in quantitative detection of mRNA expression, it may not provide information about qualitative differences, as exemplified by the anatomic distribution of the mRNA. Therefore, additional studies such as in situ hybridizations and/or proteomic studies would be required to provide a more comprehensive understanding of the neurobiological effects of the SSRI treatment on the HPA axis in this LBW model.

In accord with our previous findings, the present studies show that the 70-day-old LBW rats were insulin resistant, as reflected by both reduced ISI and reduced insulin-stimulated rates of whole body glucose uptake. The increased hepatic PEPCK mRNA levels during the OGTT strongly suggest hepatic insulin resistance and increased postprandial rates of gluconeogenesis (17, 47). In addition, basal hepatic G-6-Pase mRNA levels also tended to be upregulated, consistent with hepatic dysregulation of glucose metabolism.

In addition to the hepatic insulin resistance, we also found that the LBW rats had muscle-specific insulin resistance, as determined by the 2-[1-14C]deoxyglucose technique. This could be accounted for by an ~50% decrease in rates of insulin-stimulated glucose uptake in RG muscle, whereas glucose uptake in fat tissue and WG muscle appeared to be normal. Total GLUT4 content in RG was similar to the Cx, suggesting defects at the level of insulin signaling possibly due to decreased phosphatidylinositol 3-kinase activation, as has been shown in other conditions of muscle insulin resistance (27). Defects in insulin signaling in the muscle at the level of PKB, which is involved in initiating translocation of GLUT4, are possible given the strong trend toward decreased insulin stimulation of PKB Ser473 phosphorylation in RG muscle in LBW (LBW: 79% increase vs. Cx: 135% increase).

We have shown previously that hepatic insulin resistance is the prominent phenotype of 40-day-old rats in the present LBW model (17), and the current studies show that additional muscle insulin resistance develops later in the lives of these LBW rats. In contrast, muscle insulin resistance appears to be the most consistent finding in humans born with LBW (31, 33). Although additional hepatic insulin resistance has been suggested (15, 66), it is still unclear whether humans born with LBW have increased activity of hepatic gluconeogenesis.

ESC treatment of LBW rats completely restored whole body insulin sensitivity, as reflected by an augmented ISI as well as by normalization of insulin-stimulated glucose disposal during the clamp. Additionally, ESC treatment completely normalized hepatic postprandial PEPCK expression and basal G-6-Pase expressions, indicating normalization of hepatic gluconeogenesis in the ESC-treated LBW rats.

ESC administration improved glucose uptake and insulin stimulation of red muscle PKB Ser473 phosphorylation, indicating that ESC treatment specifically improved the defects in insulin signaling in red muscle of the LBW rats. The ESC treatment did not influence basal muscle glycogen concentrations or GLUT4 content in red or white muscle tissue.

In animal models, it is well established that, by increasing the expression of PEPCK and to a lesser extent G-6-Pase, corticosteroids are potent activators of hepatic gluconeogenesis (7, 29). Moreover, corticosteroids are known to cause insulin resistance in skeletal muscle by reducing both insulin-stimulated Ser473 and Thr608 PKB phosphorylation (18, 60). Furthermore, rat skeletal muscles exposed to high-dose dexamethasone have elevated GLUT4 protein levels despite reduced GLUT4 translocation to the cell surface (76, 77). These observations are in agreement with our findings, which strongly suggest impaired glucose transport in RG muscle tissue, possibly due to impaired PKB activation, although this was not significant. In accord with this, our LBW rats have a chronic low-grade corticosteroid overexposure, which may explain the less pronounced phenotype compared with the effects observed in skeletal muscle tissue exposed to high-dose dexamethasone (76, 77). Nevertheless, the ESC administration normalized circulating corticosteroid levels and enhanced insulin sensitivity in LBW rats, indicating a possible regulatory role for adrenal steroids for muscle insulin signaling in the LBW rats.

Although HPA axis hyperactivity may be responsible for the insulin resistance in LBW, alterations in HSD-1 (and GR)
expression in peripheral tissues may also contribute (28, 45). Increased splanchnic cortisol production [especially due to hepatic HSD-1 activity (9, 10)] has been suggested to play a role for the development of insulin resistance and obesity in humans (46, 72). In the present study, HSD-1 expression in peripheral tissues was not measured, but in the same LBW model, Nyirenda et al. (47) found no change in HSD-1 expression in liver tissues of adult rats. Furthermore, the present LBW model displays no or only modest changes in hepatic GR mRNA expression (17, 47). Although minor changes in peripheral glucocorticoid signaling may contribute to the insulin resistance in the present LBW model, the demonstrated HPA axis hyperactivity is still likely to be of greater importance.

In some studies, SSRIs cause obesity and hypercholesterolemia, whereas in others, SSRIs have been reported to exert no or even positive metabolic effects (1, 38, 56). To assess whether the metabolic changes observed in the SSRI-LBW rats could represent a direct drug effect independent of the HPA axis and LBW, a pilot study was performed to determine the effects of ESC in the Cx rats. We found that ESC treatment decreased insulin sensitivity as measured by the ISI index and plasma insulin levels and tended to increase corticosterone release during stress. Although these findings are puzzling, it clearly suggests a differentiated and phenotype-dependent drug response. Similar mechanisms might explain the inconsistent results from previous studies regarding the metabolic effects of SSRI compounds. Although further studies are needed to clarify these issues, our pilot data strongly indicate that the metabolic alterations in liver and muscle of the SSRI-LBW rats are unlikely to represent a primary drug effect; instead, these changes seem to be secondary to the reduction of HPA axis activity.

In conclusion, our data support the hypothesis that insulin resistance associated with LBW is caused by HPA axis hyperactivity and resulting elevation of corticosteroid levels. These studies are the first to demonstrate that an SSRI compound downregulates the increased HPA axis activity in adult rats born with LBW and reverses the resulting insulin resistance in both liver and muscle.

Present pharmaceutical approaches focus on a possible primary defect involved in LBW insulin resistance. Although the SSRI compounds of today might not be considered suitable for the treatment of LBW humans, other more appropriate HPA axis modulating pharmaceuticals might instead prove to be useful for the future treatment of HPA axis disturbances in humans born with LBW. Hence, this treatment strategy may reduce the risk for developing type 2 diabetes and in addition prevent the progression of other LBW-related disorders such as melancholic depression, which is known to be overrepresented in humans born with LBW.

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Present address of E. S. Buhl: Dept. of Internal Medicine, Regional Hospital Silkeborg, Central Denmark Region, Silkeborg, Denmark.

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DISCLOSURES

The authors have nothing to declare.

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E928

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