11-Dehydrocorticosterone Causes Metabolic Syndrome, Which Is Prevented when 11β-HSD1 Is Knocked Out in Livers of Male Mice

Erika Harno, Elizabeth C. Cottrell, Brian G. Keevil, Joanne DeSchoolmeester, Mohammad Bohlooly-Y, Harriet Andersén, Andrew V. Turnbull, Brendan Leighton, and Anne White

Metabolic syndrome is increasing in importance as the cluster of component conditions, obesity, type 2 diabetes, insulin resistance and hypertension become more prevalent. Many clinical symptoms of metabolic syndrome are found in patients with Cushing’s syndrome, implicating glucocorticoids (Gcs) in the etiology of this disorder (1). However, circulating cortisol levels are not elevated in obesity (2) and do not appear to be increased in metabolic syndrome (3).

The effect of exogenous Gcs on metabolic parameters in animal models has given conflicting results. In rats, corticosterone administration reduced body weight (4–6),
contrary to what would be expected from studies in adrenalectomized rodents (7, 8). Similar results have been observed in mice, where administration of Gcs in drinking water led to a loss in body weight, although their insulin levels were increased (9). However, when a higher dose of corticosterone was administered, which increased levels of circulating corticosterone to those seen during stress, this resulted in a metabolic syndrome–like phenotype in the mice, with increased body weight, adiposity, and insulin levels (10).

Although active Gcs (cortisol in humans and corticosterone in rodents) in the circulation are primarily derived from the adrenal gland as part of the hypothalamic-pituitary-adrenal (HPA) axis, they can also be regenerated from their substrate (cortisone/11-dehydrocorticosterone [11-DHC]) by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (11). This enzyme is highly expressed in metabolically active tissues, including liver and adipose tissue (12, 13), where local Gc regeneration and action can drive hepatic insulin resistance (14, 15) and promote fat accumulation (15).

However, it is not clear whether Gcs regenerated by 11β-HSD1 in tissues and acting within these tissues contribute to metabolic syndrome or whether the circulating Gcs play a more important role. Therefore, to address these mechanisms, we administered the inactive Gc, 11-DHC, to mice with a liver-specific knockout of 11β-HSD1 (LKO) and to wild-type (WT) mice. LKO mice treated with 11-DHC failed to develop the metabolic syndrome–like phenotype seen in WT mice. This suggests that loss of 11β-HSD1 from the liver prevents the development of insulin resistance and body weight gain. To prove that 11β-HSD1 was responsible for the tissue-derived corticosterone and therefore also the adverse metabolic effects, we treated 11β-HSD1 global knockout (GKO) mice with 11-DHC. These GKO mice were unaffected by 11-DHC, with no increases in corticosterone. In addition, GKO mice had no increases in body weight, adiposity, or insulin resistance. Taken together, these data highlight the importance of corticosterone regenerated in liver by 11β-HSD1 and acting within the liver for the metabolic effects of Gcs.

**Materials and Methods**

**Breeding of liver-specific and global 11β-HSD1 knockout mice**

The HSD11B1 gene targeting vector was prepared from a 129/Sv bacterial artificial chromosome clone (ResGen; Invitrogen, Paisley, United Kingdom). DNA fragments of 5.2, 1.1, and 2.6 kb (for 5′ homology, deletion, and 3′ homology regions, respectively) were cloned into a modified loxP floxed PGKneo plasmid, linearized, and electroporated into R1 mouse embryonic (ES) cells. The deletion fragment includes exon 2 (Supplemental Figure 1A, published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org). Candidate ES cell clones were screened by PCR and confirmed by Southern blotting (Supplemental Figure 1B) to identify recombinant recombination. Targeted ES cells were injected into C57BL/6 blastocysts and embryos implanted into pseudopregnant B6CBA female mice. Chimeric animals were mated with C57BL/6 to produce agouti heterozygous animals (F1). For generating 11β-HSD1 conditional (floxed) mice, the heterozygous F1 mice were bred with Ella-Cre transgenic mice (C57BL/6 background) to delete the loxP floxed PGKneo cassette. The primers used to detect the WT allele (315 bp) or floxed allele (440 bp) were as follows: forward: TATCTGCCCACCTTGTCCCTCTT and reverse: CCATGAGCTTTCCCGCGAC, respectively.

Cre/loxP technology was used to generate mice with liver-specific disruption of the HSD11B1 gene. Male mice heterozygous for the conditional HSD11B1 allele and Cre recombinase under the control of the rat albumin promoter were mated with female homozygous conditional HSD11B1 mice. The resulting Cre-positive, 11β-HSD1lox/lox offspring (LKO) and Cre-negative 11β-HSD1lox/lox (WTf/f) were determined by genotyping at weaning. The Cre status of mice was determined in each strain using the following primers: forward: ATGAAATGCCACCTATTGCCTCTT and reverse: CCATGAGCTTTCCCGCGAC, giving a band size of 565 bp in Cre-positive (LKO) and no band in floxed (WTf/f) mice.

For generation of GKO mice, heterozygous F1 males were bred with Rosa26-Cre transgenic mice (C57BL/6 background) to delete the loxP floxed region including exon 2 and the neo cassette, and 11β-HSD1 null mice for studies were obtained by intercrossing. Offspring were genotyped at weaning by PCR using specific primers to detect WT allele (1.6-kb fragment) and null allele (0.6-kb fragment), respectively: forward: CAGGTTAGTACCCTGTTGCCCA and reverse: AGTCCGCCTGCAAAGAGATAGTG.

**Quantitative mRNA analysis**

Total RNA was extracted from brain, liver, and epididymal adipose tissue using either Trizol reagent (brain and adipose tissues; Invitrogen) or RNaseasy mini kit (liver; Qiagen, Manchester, United Kingdom), according to the manufacturer’s instructions. Contaminating genomic DNA was removed using a TurboDNAse kit (Ambion, Paisley, United Kingdom). RNA quantity and integrity were determined using a NanoDrop (ND1000; NanoDrop Technologies, Wilmington, Delaware), before one-step quantitative RT-PCR was carried out using Taqman RNA-to-C_{T} 1-Step Kit (Applied Biosystems, Paisley, United Kingdom) for the measurement of HSD11B1 mRNA expression. Specific Taqman probes were used to amplify a region of exon 1 and 2 of the HSD11B1 gene (Mm01313990_m1), and values were normalized to levels of hypoxanthine-guanine phosphoribosyltransferase (Mm00446968_m1), using standard curve analysis.

**11β-HSD1 activity assay**

11β-HSD1 enzyme activity was determined in vitro, adapted from a method by Hermanowski-Vosatka et al (16), by measuring conversion of 3H-cortisone to 3H-cortisol. Tissues (approximately 100 mg) were incubated for 10 minutes (liver), 45 minutes (brain), or 60 minutes (adipose) in the presence of 3H-cortisone (20 nmol/L, 1 μCi/mL, specific activity 1.97 GBq/
mmol; PerkinElmer, Waltham, Massachusetts) containing DMEM Ham F12 media (Sigma-Aldrich, Dorset, United Kingdom) supplemented with 10% fetal calf serum and 1% penicillin/streptomycin in 37°C at 5% CO₂. After tissue incubations, medium samples were collected for analysis of cortisone to cortisol conversion. Radiolabeled steroids were extracted using ethyl acetate; samples were evaporated to dryness under nitrogen and resuspended in mobile phase for HPLC analysis (methanol:H₂O, 50:50), adapted from Napolitano et al (17). Radiolabeled steroids were separated using reversed-phase HPLC, Agilent 1200 HPLC using a Kromasil C18 5-μm column, 4.6 mm × 250 mm (Crawford Scientific, Lanarkshire, United Kingdom) with methanol:H₂O (50:50) at flow rate of 1.5 mL/min. Radioactivity was measured using a flow scintillation analyzer (Radiomatic series 500TR; PerkinElmer Analytical Instruments) with FLO-ONE software. 11β-HSD1 activity was expressed as percentage conversion per 100 mg tissue.

Experimental design

Male LKO, GKO, and their WT littermates (WT* and WT, respectively) were singly housed throughout the study. They were maintained on a 12-hour light, 12-hour dark cycle (lights on at 0600 h) and fed standard chow (RM1; Special Diet Services, Essex, United Kingdom) ad libitum. Mice were administered 11-DHC (Stratech Scientific, Suffolk, United Kingdom) in drinking water at doses of 25 μg/mL (LKO and GKO) or 50 μg/mL (GKO) or vehicle (1% ethanol) for 5 weeks. Mice were weighed daily and food intake was measured weekly during the study. On day 29, mice were underwent an oral glucose tolerance test (OGTT). Blood samples were taken by tail venesection microsampling and, due to the small sample volume, repeat measurements were made in accordance with the UK Home Office Animals (Scientific Procedures) Act 1986 and approved by the local Ethical Review Committee.

OGTT

Mice were fasted for 16 hours before baseline blood glucose was measured (Accu-cheek glucometer; Roche Diagnostics, West Sussex, United Kingdom) and blood samples (5 μL) were collected for insulin measurement (Crystal Chem ELISA, Downers Grove, Illinois). Mice were administered 10 mL/kg of 20% glucose by oral gavage (0 min) and glucose measurements were taken at 20, 40, 60, and 90 minutes. Blood samples were collected for insulin measurements at 20 and 40 minutes. All blood samples were taken under terminal CO₂/O₂ narcosis. These time points are 1 hour into the light phase and 1 hour into the dark phase at the previously determined nadir and peak of corticosterone measurements on day 34, and on day 35 terminal blood samples were taken under terminal CO₂/O₂ narcosis. This was measured (Accu-chek glucometer; Roche Diagnostics, West Sussex, United Kingdom) and blood samples (5 μL) were collected for insulin measurement (Crystal Chem ELISA, Downers Grove, Illinois). As appropriate, t tests, one-way or two-way ANOVAs with Bonferroni post-hoc tests were used. P < .05 was considered significant.

Results

11-DHC administration increases corticosterone in WT mice

To determine whether exogenously administered 11-DHC could be converted to corticosterone and enter the circulation, mice were administered 25 μg/mL or 50 μg/mL 11-DHC in drinking water for 5 weeks. These concentrations of 11-DHC led to dose-dependent increases in circulating corticosterone at both nadir (Figure 1A) and peak (Figure 1B). Circulating levels of 11-DHC are difficult to measure, as orally administered 11-DHC is metabolized or excreted within 15 minutes (20). Therefore the doses of 11-DHC that were chosen resulted in an elevation of circulating corticosterone to levels similar to those seen after restraint stress (21, 22).

Treatment with 11-DHC down-regulates HPA axis biomarkers

We investigated the effect of 5 weeks of chronic 11-DHC administration on HPA axis biomarkers. Administration of either dose of 11-DHC decreased adrenal gland weight in WT mice compared to vehicle controls (Figure 1C). Circulating ACTH levels were also decreased in response to the increased circulating corticosterone levels at both the nadir (Figure 1D) and the peak (Figure 1E). The precursor of ACTH, POMC, was also decreased by 11-DHC in these mice, which is an indication of POMC gene suppression (Figure 1F).

11-DHC can induce a metabolic syndrome–like phenotype in mice

To investigate the effects of 11β-HSD1-derived corticosterone, metabolic parameters including body weight and glucose and insulin responses were examined after chronic 11-DHC treatment. By study day 28, WT mice treated with 50 μg/mL 11-DHC had increased percentage body weight compared to vehicle controls (Figure 2A) and also had higher body weight gain (Figure 2B). This was coupled with an increase in food intake (Figure 2C) and an
increase in epididymal fat pad mass (Figure 2D). Similar increases were also seen in mesenteric and subcutaneous fat pad masses with 50 \( \mu \)g/mL 11-DHC (data not shown).

11-DHC (50 \( \mu \)g/mL) also increased fasting insulin and insulin excursion from basal levels following oral glucose administration (Figure 2E). There was also a trend toward an increase in both these parameters in the 25 \( \mu \)g/mL 11-DHC group (Figure 2E). Homeostatic Model of Assessment–Insulin Resistance (HOMA-IR), a measure of insulin resistance, was also increased with 50 \( \mu \)g/mL 11-DHC (Figure 2F). There was no difference in glucose excursion during an OGTT between any of the treatment groups (Figure 2G).

LKO mice do not develop a metabolic syndrome–like phenotype with 11-DHC treatment

As liver is the primary site of 11\( \beta \)-HSD1 expression, we developed a mouse model with 11\( \beta \)-HSD1 knocked-out only in the liver (LKO; Supplemental Figure 1A). These mice had a greater than 90% reduction in mRNA expression and enzyme activity only in liver, with no effect on the expression or activity in brain or adipose tissue compared to their WT floxed littermates (WT\( ^{f/f} \); Supplemental Figure 2, A and B). LKO mice had no metabolic or HPA axis phenotype (Supplemental Figure 2, C–I) and as reported previously (23).

To investigate the role of hepatic 11\( \beta \)-HSD1 on metabolic parameters we chronically administered 11-DHC to LKO mice, which do not have the capacity to convert 11-DHC to corticosterone in liver, but have normal 11\( \beta \)-HSD1 activity in other tissues. Unlike WT\( ^{f/f} \) mice, LKO mice did not have increased body weight with 11-DHC treatment (Figure 3A). There was no change in epididymal fat pad mass in LKO mice treated with 25 \( \mu \)g/mL 11-DHC, whereas it was increased in the WT\( ^{f/f} \) mice (Figure 3B). In addition, neither strain had increased food intake in response to 25 \( \mu \)g/mL 11-DHC (Figure 3C).

LKO mice were protected from the 11-DHC-induced hyperinsulinemia seen in WT\( ^{f/f} \) mice. In response to glucose, LKO mice treated with 25 \( \mu \)g/mL 11-DHC were indistinguishable from vehicle-treated WT\( ^{f/f} \) (Figure 3D) or LKO mice (Figure 3E). However, neither WT\( ^{f/f} \) nor LKO mice had any changes in glucose tolerance during an OGTT (Figure 3G).

11-DHC administration increases corticosterone and down-regulates the HPA axis in LKO mice

To investigate whether the lack of metabolic changes in LKO mice were due to an absence of increased circulating corticosterone or to inhibition of conversion of 11-DHC to corticosterone only in the liver, we measured circulating corticosterone in these mice. Chronic treatment with 25 \( \mu \)g/mL 11-DHC increased circulating corticosterone in both LKO and their WT\( ^{f/f} \) littermates at both nadir and peak (Figure 4, A–D). This resulted in concentrations of corticosterone in both strains that were similar to levels seen during stress.

To investigate whether circulating concentrations of corticosterone were responsible for the down-regulation
of the HPA axis, we measured biomarkers of the axis in both LKO and WTf/f mice. Both strains of mice had a similar magnitude of down-regulation of the HPA axis in response to chronic 11-DHC treatment and in turn raised circulating corticosterone. Both LKO and WTf/f mice had a similar reduction in adrenal gland weight (Figure 4E).

ACTH was decreased in response to 25 μg/mL 11-DHC in WTf/f mice at both nadir (Figure 4F) and peak (Figure 4G); in LKO mice, however, the decrease in ACTH was only significant at the peak (Figure 4G). POMC was also decreased in WTf/f and LKO mice treated with 11-DHC (Figure 4H).

Global 11β-HSD1 knock-out mice are unaffected by 11-DHC treatment

To test the hypothesis that 11-DHC treatment increased circulating corticosterone in WT mice solely through regeneration by 11β-HSD1, we developed 11β-HSD1 GKO. These mice had exon 2 of the HSD11B1 gene removed (Supplemental Figure 1A) and were shown to have a greater than 92% reduction in 11β-HSD1 mRNA and enzyme activity in brain, liver, and adipose tissue (Supplemental Figure 3, A and B). The GKO mice were similar in body weight, glucose, and insulin responses to their WT littermates when on a chow diet (Supplemental Figure 3, C–E) as seen previously (24). Global knockout of 11β-HSD1 increased adrenal gland weight, as is commonly observed (19, 25), but had no effect on other HPA axis parameters including circulating corticosterone when compared to WT littermates (Supplemental Figure 3, F–I).

In the GKO mice, corticosterone (which must be derived from the adrenal gland) was similar to that in the WT mice. Chronic 11-DHC treatment with either 25 μg/mL or 50 μg/mL 11-DHC did not increase circulating corticosterone at either nadir (Figure 5A) or peak (Figure 5B). GKO mice also did not have any changes in HPA axis biomarkers with either 25 μg/mL or 50 μg/mL 11-DHC. There was no change in adrenal gland weight between the treatment groups (Figure 5C) as well as no difference in circulating ACTH levels at either nadir (Figure 5D) or peak (Figure 5E). POMC was not different between the groups (Figure 5F).

Similar results were seen in metabolic parameters. GKO mice had no increase in body weight (Figure 6A) or epididymal fat pad mass (Figure 6B), nor in mesenteric or subcutaneous fat pad masses (data not shown). All the treatment groups had similar food intake (Figure 6C). During an OGTT, vehicle and 11-DHC-treated mice had similar glucose (Figure 6D) and insulin (Figure 6E) responses and a similar HOMA-IR value (Figure 6F).

Discussion

The involvement of Gcs in metabolic syndrome has been the subject of much debate. Although circulating Gcs are

Figure 2. Chronic 11-DHC treatment causes a metabolic syndrome–like phenotype in WT mice. (A) Percentage body weight change over 28 days of treatment. (B) Absolute body weight change. (C) Percentage change in food intake after 4 weeks of 11-DHC treatment compared to baseline. (D) Relative epididymal fat pad mass as a percentage of body weight (% BWT). (E) Insulin excursion during an OGTT. (F) HOMA of insulin resistance. (G) Glucose excursion during an OGTT from WT mice treated with vehicle (1% ethanol), 25 μg/mL, or 50 μg/mL 11-DHC. White circle, vehicle; gray square, 25 μg/mL 11-DHC; black triangle, 50 μg/mL 11-DHC. Group sizes were 10–12. Data are expressed as mean ± SEM. *, P < .05; **, P < .01; ***, P < .001 vs vehicle-treated mice or as marked.

do: 10.1210/en.2013-1362 endo.endojournals.org 3603
not overtly elevated in patients with metabolic syndrome, it may be that the methodology is not sophisticated enough for measuring subtle increases in Gcs in the presence of intrapatient variation in pulsatility and mild stress-related responses. Nevertheless, an alternative explanation is that corticosterone regenerated by the enzyme 11β-HSD1 in tissues, and specifically the liver, may be implicated in the etiology of metabolic syndrome. This is supported by the data presented here showing that deletion of hepatic 11β-HSD1 prevented the metabolic syndrome–like phenotype associated with administration of 11-DHC. This lack of metabolic phenotype occurred in the presence of increased circulating Gcs, indicating that changes in intratissue Gc levels were more important than the alterations in circulating levels. In addition, liver 11β-HSD1 does not appear to be the source of raised circulating Gcs in response to

---

**Figure 3.** LKO mice are resistant to the metabolic effects of 11-DHC treatment. (A) Percentage body weight change on day 28 in LKO and floxed WT control (WTf/f) mice. (B) Relative epididymal fat pad mass expressed as a percentage of body weight (%BWT). (C) Average weekly food intake over 4 weeks of 11-DHC treatment. (D) Insulin excursion during an OGTT in WTf/f and LKO mice. (E) HOMA of insulin resistance. (G) Glucose excursion during an OGTT in WTf/f and LKO mice treated with vehicle (1% ethanol) or 25 μg/mL 11-DHC. **Black circle**, vehicle-treated WTf/f; **black square**, 25 μg/mL treated WTf/f; **white circle**, vehicle-treated LKO; **white square**, 25 μg/mL 11-DHC-treated LKO. Group sizes were 11–14. Data are expressed as mean ± SEM. **, P < .01; ***, P < .001 vs vehicle-treated mice of the same genotype.
11-DHC treatment, but instead the most likely origin is adipose tissue. All effects of 11-DHC were absent in GKO mice, indicating the crucial role of 11β-HSD1 in the development of metabolic syndrome. The role of Gcs in modulating metabolic parameters predicts that subtle changes in Gc production or action could predispose to metabolic syndrome. Indeed, it is well known that Gcs can affect adiposity, promoting increased fat mass by stimulating adipose tissue hyperplasia, through increased storage of lipids, and hypertrophy, by increased differentiation of stromal cells and preadipocytes (26), as well as by redistribution to visceral stores. However the increase in fat pad mass and body weight in this study after chronic 11-DHC treatment in WTf/f mice occurred in the absence of increases in food intake. Therefore this increase in body weight is most likely due to decreased energy expenditure, although this was not measured in the present study. The reduced energy expenditure could be attributed to mild myopathy of the skeletal muscle and recent studies have shown that Gc administration can lead to increased markers of muscle atrophy together with decreased muscle strength (27).

Figure 4. LKO mice have elevated circulating corticosterone and a reduction in HPA axis biomarkers after chronic 11-DHC treatment. (A) Circulating corticosterone levels measured in plasma samples taken by tail venesection in floxed WT littermates (WTf/f) mice at nadir (7 AM) and (B) at peak (7 PM), and (C) in LKO mice at nadir (7 AM) and (D) at peak (7 PM). (E) Adrenal gland weight. (F) Plasma ACTH levels measured in terminal samples after CO2/O2 narcosis at nadir (7 AM) and (G) at peak (7 PM). (H) Circulating POMC measured in terminal samples from WTf/f or LKO mice treated with vehicle (1% ethanol) or 25 µg/mL 11-DHC. Group sizes were 10–14, except for ACTH, where they were 5–7. Data are expressed as mean ± SEM. *, P < .05; **, P < .01; ***, P < .001 vs vehicle-treated mice of the same genotype.
tive explanation could be that Gc administration led to decreased activity in the mice (10), which may also account for the increased body weight in the absence of increased food intake.

The effect of Gcs on hepatic glucose production (28), where Gcs have direct effects on PEPCK and PGC-1/H9251 gene expression (29), gives clear evidence for increased glucose output, which would be counteracted initially by hyperinsulinemia (30, 31). However, direct effects of Gcs on insulin receptor signaling pathway elements, such as insulin receptor, and insulin receptor substrates 1 and 2, may also be responsible for Gc-induced insulin resistance (32, 33). Therefore, the action of 11-DHC-derived corticosterone on these metabolic parameters is predictable. In addition, in mice where corticosterone administration in drinking water raised circulating corticosterone to levels similar to those seen in the present study (10), the mice had increased adiposity, body weight gain, and hyperinsulinemia, consistent with our findings.

The data presented here indicate that administration of the substrate for 11β-HSD1 is capable of increasing corticosterone and generating a metabolic syndrome–like phenotype. Surprisingly, knockout of 11β-HSD1 specifically in liver was sufficient to prevent these metabolic changes. Despite the circulating corticosterone concentrations in LKO and WTf/f mice being similar, the decreased 11β-HSD1-derived corticosterone within the liver was sufficient to prevent body weight gain. It has been assumed that the body weight effects of Gcs are due to changes in circulating levels, supported by the fact that adrenalectomy reduces circulating corticosterone levels and also reverses obesity (7, 8). However, adrenalectomy also prevents corticosterone produced in the adrenal being converted to 11-DHC in the kidney and so removes the substrate for 11β-HSD1. Therefore, from the data presented here, it appears that it is the intratissue-generated corticosterone levels that are important for body weight gain, rather than the whole-body circulating concentrations. Moreover, adipose tissue and brain are the main sites of 11β-HSD1 activity generally associated with regulation of body weight and adiposity. However, the lack of body weight gain and increased epididymal fat pads in LKO mice treated with 11-DHC suggest corticosterone regenerated in liver is also important for adipose tissue deposition and fat accumulation.

In the present study, WT mice treated with 11-DHC had markedly increased fasting insulin and an increased insulin response during a glucose challenge. This result was consistent with other models where Gc-induced peripheral insulin resistance leads to compensatory β-cell proliferation and increased insulin secretion (30, 31). However, no differences in glucose were observed between control and 11-DHC-treated mice, suggesting that at the stage examined, β-cell compensation was sufficient to maintain normoglycemia. In addition, the increased insulin response was not observed in GKO mice, indicating that either the tissue-regenerated corticosterone or the resulting increases in circulating corticosterone were responsible for the reduction in insulin sensitivity in WT
mice. This was clarified using LKO mice, where circulating corticosterone was increased but there was no change in insulin sensitivity. This provides evidence that 11β-HSD1-derived corticosterone in liver has a role in Gc-induced hyperinsulinemia. Taken together, these data suggest that 11-DHC treatment leads to relative hyperinsulinemia in WT mice, and this maintains normoglycemia during a glucose challenge.

There is still some uncertainty about the contribution of 11β-HSD1-derived Gcs to circulating concentrations of Gcs. In human subjects, deuterated tracers have been used to measure 11β-HSD1 contribution from liver and adipose tissue. These tracers were detected in the portal vein (34, 35), indicating that regenerated Gcs enter the circulation at least locally, but whole-body measurements have not been determined. A mouse model of adipose tissue

Figure 6. Chronic 11-DHC treatment does not adversely affect metabolic parameters in GKO mice. (A) Percentage body weight change over 28 days of treatment. (B) Relative epididymal fat pad mass as a percentage of body weight (% BWT). (C) Percentage change in food intake after 4 weeks of treatment compared to baseline. (D) Glucose and (E) insulin excursion during an OGTT. (F) HOMA of insulin resistance from GKO mice treated with vehicle (1% ethanol), 25 μg/mL, or 50 μg/mL 11-DHC. White circle, vehicle (1% ethanol); gray square, 25 μg/mL 11-DHC; black triangle, 50 μg/mL 11-DHC. Group sizes were 10–12. Data are expressed as mean ± SEM.
11β-HSD1 overexpression exhibited no increase in whole-body circulating corticosterone levels (15). However, increased portal vein concentrations were observed, which could be attributed to increased adipose tissue regeneration and local release. The current study provides evidence that active Gcs can enter the blood, as WT mice treated with 11-DHC have increased plasma corticosterone levels in the presence of a decreased HPA axis response. Furthermore, adipose tissue is most likely to be the main site of production of this 11β-HSD1-derived corticosterone, as LKO mice still have increased circulating corticosterone in response to 11-DHC in the absence of liver 11β-HSD1. Adipose tissue would be the only other organ with sufficient 11β-HSD1 to regenerate the observed levels of corticosterone. Thus, an additional key finding of the present study is that corticosterone regenerated from increased 11-DHC levels by 11β-HSD1, most likely in adipose tissue, enters the circulation resulting in markedly increased plasma levels.

Treatment with 11-DHC led to a reduction in HPA axis activity in WT mice at least in terms of decreases in adrenal gland weight, circulating POMC, and ACTH. This result is compatible with increases in circulating corticosterone leading to down-regulation of the HPA axis via a negative feedback loop (11). Similarly, LKO and WTf/f mice treated with 11-DHC had comparable increases in circulating corticosterone and also had parallel reductions in adrenal gland weight, POMC, and ACTH. Vehicle-treated GKO mice had similar corticosterone to WT littermates as is commonly seen in mice bred on a C57BL/6 background (19, 36–39). This is however different to the original GKO mice bred on a 129 background, where increased circulating corticosterone was noted in the knock-out mice (21, 25, 40). As expected, in this study 11-DHC did not raise circulating corticosterone and did not cause a reduction in the HPA axis in mice with a global knockout of 11β-HSD1.

In conclusion, this study has shown that inhibition of corticosterone regeneration from 11-DHC by liver 11β-HSD1 can protect mice from glucocorticoid-induced metabolic syndrome. Chronic administration of 11-DHC led to pathophysiological symptoms, including increased body weight gain, adiposity and food intake, as well as hyperinsulinemia. Studies in mice with knockout of 11β-HSD1 in liver have indicated a key role for liver intratissue corticosterone in these metabolic changes. Finally, LKO mice treated with 11-DHC had increased circulating corticosterone levels, indicating that the major source of 11β-HSD1-derived corticosterone that enters the circulation is generated elsewhere. This novel model suggests that tissue-derived Gcs may be important in contributing to whole-body metabolic dysfunction.

Acknowledgments

The authors thank AstraZeneca and the University of Manchester Biomedical Research Centre for their funding and support during the studies. Grateful thanks are also given to David Tucker, Lynne Vicary, and the transgenic team at AstraZeneca for the production and genotyping of the 11β-HSD1 KO mice, to Bill Brown and the IPG-Animal Science and Welfare support team at AstraZeneca for the generation of experimental results, and to Gemma Convey, Julie Cook, and Usha Chauhan at AstraZeneca for analytical support.

Address all correspondence and requests for reprints to: Prof. Anne White, Faculty of Life Sciences, AV Hill Building, University of Manchester, Manchester, M13 9PT, United Kingdom. E-mail: awhite@manchester.ac.uk.

Disclosure Summary: E.H. and A.W. receive grant funding from AstraZeneca. J.D.S., M.B.-Y., H.A., A.V.T., and B.L. are employees of AstraZeneca. The other authors have no conflicts of interest to declare.

References