JCEM THE JOURNAL OF CLINICAL ENDOCRINOLOGY & METABOLISM

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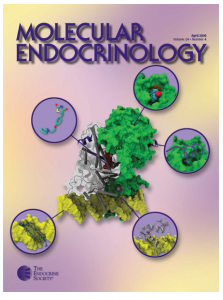
J. Clin. Endocrinol. Metab. 2010 95:5348-5356 originally published online Sep 8, 2010; , doi: 10.1210/jc.2010-0729



Cerebrospinal Fluid Corticosteroid Levels and Cortisol Metabolism in Patients with Idiopathic Intracranial Hypertension: A Link between 11β-HSD1 and Intracranial Pressure Regulation?

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Context: The etiology of idiopathic intracranial hypertension (IIH) is unknown. We hypothesized that obesity and elevated intracranial pressure may be linked through increased 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) activity.

Objective: The aim was to characterize 11β-HSD1 in human cerebrospinal fluid (CSF) secretory [choroid plexus (CP)] and drainage [arachnoid granulation tissue (AGT)] structures, and to evaluate 11β-HSD1 activity after therapeutic weight loss in IIH.

Design and Setting: We conducted *in vitro* analysis of CP and AGT and a prospective *in vivo* cohort study set in two tertiary care centers.

Patients or Other Participants: Twenty-five obese adult female patients with active IIH were studied, and 22 completed the study.

Intervention: Fasted serum, CSF, and 24-h urine samples were collected at baseline, after 3-month observation, and after a 3-month diet.

Main Outcome Measures: Changes in urine, serum, and CSF glucocorticoids (measured by gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry) after weight loss were measured.

Results: 11β-HSD1 and key elements of the glucocorticoid signaling pathway were expressed in CP and AGT. After weight loss (14.2 $\pm$ 7.8 kg; $P < 0.001$), global 11β-HSD1 activity decreased ($P = 0.001$) and correlated with reduction in intracranial pressure ($r = 0.504$; $P = 0.028$). CSF and serum glucocorticoids remained stable, although the change in CSF cortisone levels correlated with weight loss ($r = -0.512$; $P = 0.018$).

Conclusions: Therapeutic weight loss in IIH is associated with a reduction in global 11β-HSD1 activity. Elevated 11β-HSD1 may represent a pathogenic mechanism in IIH, potentially via manipulation of CSF dynamics at the CP and AGT. Although further clarification of the functional role of 11β-HSD1 in IIH is needed, our results suggest that 11β-HSD1 inhibition may have therapeutic potential in IIH. **(*J Clin Endocrinol Metab* 95: 5348–5356, 2010)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-0729 Received March 26, 2010. Accepted August 5, 2010.
First Published Online September 8, 2010
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Abbreviations: AGT, Arachnoid granulation tissue; An, androsterone; BMI, body mass index; CP, choroid plexus; CSF, cerebrospinal fluid; Em, total cortisone metabolite; Et, etiocholanolone; Fm, total cortisol metabolite; GE, glycyrrhetinic acid; GR, glucocorticoid receptor; H6PD, hexose-6-phosphate dehydrogenase; 11β-HSD, 11β-hydroxysteroid dehydrogenase; ICP, intracranial pressure; IIH, idiopathic intracranial hypertension; LC/MS, liquid chromatography/tandem mass spectrometry; MR, mineralocorticoid receptor; sgk, serum and glucocorticoid-regulated kinase; THE, tetrahydrocortisone; THF, tetrahydrocortisol; VLCD, very low-calorie diet.

Idiopathic intracranial hypertension (IIH), also known as benign intracranial hypertension or pseudotumor cerebri, is a disabling condition among young women, characterized by elevated intracranial pressure (ICP) in the absence of cerebral space occupation or venous sinus thrombosis (1). Affected individuals experience headaches and papilledema, with up to 25% reporting sustained visual loss (2). The etiology of IIH is not known, although disordered cerebrospinal fluid (CSF) dynamics, with either enhanced CSF production at the choroid plexus (CP) or restricted CSF drainage at the arachnoid granulation tissue (AGT), are fundamental (3). Of particular interest is that over 90% of patients with IIH are obese (4), the incidence of IIH in an obese population being 20 per 100,000 (background incidence, 2 per 100,000; from the World Health Organization Global Database on Body Mass Index: http://apps.who.int/bmi/index.jsp). In conjunction with the global epidemic of obesity, the prevalence of IIH is rising, contributing to significant morbidity in young women.

The link between IIH and obesity is not explained. Previous theories implicating obstructive sleep apnea or pressure effects from centrally distributed adiposity have not been substantiated (6, 7). The link between corticosteroids and obesity is exemplified in patients with Cushing's syndrome. Numerous case reports have also documented the development of IIH in association with glucocorticoid therapy and Cushing's syndrome (3, 8, 9). However, Cushing's syndrome is rare, and circulating cortisol concentrations are normal in most patients with obesity and IIH. Nevertheless, cortisol metabolism is deranged in obese subjects through aberrant activity of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (10, 11). Two isoforms of 11β-HSD determine, at a prereceptor level, corticosteroid availability. 11β-HSD1, highly expressed in adipose tissue and liver, acts predominantly as an oxoreductase *in vivo* (activating cortisol from cortisone) and has key roles in regulating adipocyte differentiation and driving hepatic gluconeogenesis (12). The contrasting isoform, 11β-HSD2 (a dehydrogenase), inactivates cortisol to cortisone in mineralocorticoid target tissues such as the kidney and colon.

Overexpression of adipose 11β-HSD1 causes visceral obesity in mouse models (13). In human obesity, 11β-HSD1 activity, assessed from urinary gas chromatography/mass spectrometry (10) in conjunction with hepatic 11β-HSD1 activity (demonstrated using cortisol generation curves) (10, 11), and adipose tissue 11β-HSD1 mRNA expression (14, 15) are abnormal. Increased 11β-HSD1 activity in human sc adipose tissue, associated with altered lipid metabolism and insulin resistance (16), has implicated 11β-HSD1 in the development of the metabolic syndrome. Recombinant mice lacking 11β-HSD1 and therapeutic inhibition of the enzyme in man improves glucose tolerance and insulin resistance (17). Of note, weight loss using a very low-calorie diet (VLCD) has been shown to alter 11β-HSD1 activity and reduce glucocorticoid generation in obese patients (18, 19), although diet-specific effects upon glucocorticoid metabolism in humans have not been fully defined.

We hypothesize that 11β-HSD1 may have a central role in the pathogenesis of IIH. Weight loss has been established as an efficacious treatment in IIH (20), and this may relate to alterations in local glucocorticoid concentrations through a reduction in 11β-HSD1.

11β-HSD1 may influence the pathogenesis of IIH through regulation of ICP. CP secretion of CSF results from intracellular carbonic anhydrase activity and, ultimately, sodium transport (Na^+) by the Na^+K^+ATPase pump and epithelial sodium channels, which create an osmotic gradient to drive water into the CSF (21). An analogous mechanism occurs in the production of aqueous humor by the embryologically related ocular ciliary epithelium. Here, cortisol generation by 11β-HSD1 stimulates serum and glucocorticoid-regulated kinase (sgk) 1 to increase epithelium Na^+ transport and aqueous humor production (22). Inhibition of 11β-HSD1 lowers intraocular pressure (23). We suggest that, akin to the ocular ciliary body, in the CP CSF secretion may be regulated by 11β-HSD1 (Fig. 1). In support of this, we have previously demonstrated 11β-HSD1 activity in the New Zealand white albino rabbit CP (24).

Glucocorticoids reduce drainage of aqueous humor at the ocular trabecular meshwork as illustrated in topical dexamethasone-induced glaucoma. In the trabecular meshwork, paracrine actions of glucocorticoids, via glucocorticoid receptor α (GRα), up-regulate glucocorticoid responsive proteins, such as myocilin, which compacts the microconfiguration of the drainage tissue, consequently elevating ocular pressure (25). Regulation of CSF secretion by the AGT is poorly defined. We propose that a mechanism, comparable to that in the glucocorticoid-sensitive trabecular meshwork, may exist to limit CSF outflow and elevate ICP in IIH. We therefore aimed to characterize 11β-HSD1 in human CP and AGT and to evaluate glucocorticoid metabolism and 11β-HSD1 in a cohort of patients with IIH before and after weight loss.

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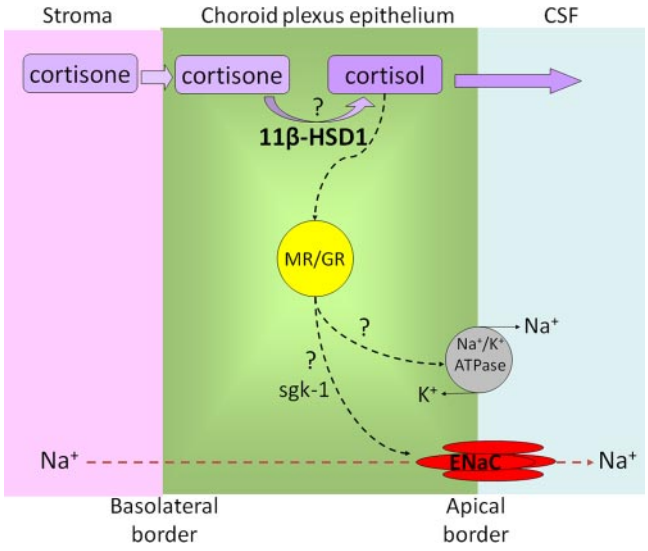

FIG. 1. Schematic diagram of the CP epithelium illustrating the proposed regulation of CSF secretion by corticosteroids. We suggest that 11β-HSD1 generation of cortisol, with subsequent up-regulation of sgk-1, stimulates epithelial sodium transport. The net movement of sodium ions (Na^+) into the cerebral ventricle is known to create an osmotic gradient to drive CSF secretion.

Materials and Methods

Tissues

Human CP and AGT were obtained from the UK Parkinson's Disease Society Brain Bank (Imperial College, London, UK) after ethical approval [Dudley Local Research Ethics Committee (LREC) 06/Q2702/19]. The AGT and CP samples were from the same patients. All donors were male patients with Parkinson's disease, none of whom had used glucocorticoid therapy, with a mean age of 75 ± 7.4 yr. Drug therapy included dopamine agonists, L-dopa and entacapone, selegiline, amitriptyline, and amantadine. Samples were initially identified using hematoxylin and eosin staining.

Immunohistochemistry

Formalin-fixed human CP and AGT (n = 6) were embedded in paraffin, and 5-μm sections were mounted on electrostatically charged slides (VWR, Lutterworth, UK). Immunohistochemical analyses were performed using in-house-generated human 11β-HSD1 (amino acids 18-33) and 11β-HSD2 (amino acids 137-160 and 334-358) antisera (Binding Site, Birmingham, UK), as previously reported (24). Human liver and kidney were used as positive control tissues for 11β-HSD1 and -2, respectively, and antibody pretreated with immunizing peptide was used as a negative control.

11β-HSD activity assay

Human CP (n = 5) and AGT (n = 2) were processed within 30 h of postmortem. Tissue was washed in PBS, surgically dissected from surrounding tissue, and divided into equal-sized pieces (approximately 100 mg). Tissue was incubated with 100 nM cortisol (to measure the dehydrogenase reaction) or 100 nM cortisone (oxo-reductase reaction) (Sigma-Aldrich Company Ltd., Dorset, UK) with tracer amounts of tritiated steroids at 37 C for 24 h as previously described (24). The 100-fold excess of glycyrrhetinic acid (GE) (5 μM) was used as a nonspecific 11β-HSD inhibitor. Enzyme activities were expressed as picomoles per gram wet weight per hour.

RNA isolation and PCR

RNA was extracted from fresh-frozen CP and AGT using the Genelute mammalian total RNA extraction kit (Sigma-Aldrich Company Ltd.) and reverse-transcribed using Multiscribe reverse transcriptase (Applied Biosystems, Warrington, UK) according to the manufacturer's protocol. RNA concentration and quality were assessed using a Nanodrop spectrophotometer (NanoDrop Products, Wilmington, DE), and standardized mRNA expression was monitored through comparison to housekeeping gene 18s. PCR was carried out using GoTaq DNA polymerase (25 ng/μl), magnesium chloride (2 mM), and deoxynucleotide triphosphate (0.25 mM) with forward and reverse primers (0.5 μM) (Promega, Southampton, UK) according to the manufacturer's guidelines. Primer sequence (Alta Biosciences, Birmingham, UK) and cycle details are listed in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Clinical study

Study population

Female subjects with IIH were identified from the Birmingham and Midland Eye Centre and University Hospitals of Leicester National Health Service Trust, United Kingdom, after informed written consent with ethical approval from Dudley LREC (06/Q2702/64). IIH subjects, in accordance with the Dandy criteria (1), had papilledema and ICP greater than 25 cm H_2O in the context of normal magnetic resonance imaging and venography. Exclusion criteria included those aged less than 16 yr, previous surgical management of IIH, pregnancy, hormone manipulating medication, and significant comorbidity including unstable diabetes and known endocrinopathies.

Study design and sample collection

Subjects were prospectively recruited into a two-stage study; stage 1 consisted of a 3-month observation period, and stage 2, a 3-month very low-calorie (425 Kcal/d) total meal replacement nutritionally complete liquid diet (VLCD) (Lipotrim; Howard Foundation, Cambridge, UK). During the VLCD, subjects consumed no additional food, drank a minimum of 2 liters of water per day, and had their weight assessed weekly. Subjects were further assessed at baseline, end of stage 1, and end of stage 2. Anthropometric data were collected at these stages, including body mass index (BMI; kilograms/meter^2) and waist circumference. Evaluation of papilledema by ultrasonographic measurement of optic disc height (20 MHz scan; Quantel Medical, Clermont-Ferrand, France) and headache, via a Headache Impact Test-6 questionnaire (26), was carried out. Samples of CSF and serum were collected between midday and 1400 h. ICP was measured by manometry before CSF collection by atraumatic, ultrasound-guided lumbar puncture. After collection, CSF and blood samples were promptly centrifuged at 176 × *g* for 10 min, aliquoted, stored at −80 C, and analyzed after a maximum of one freeze-thaw cycle. Urine samples (24 h) were also collected, and aliquots were stored at −80 C.

Gas chromatography/mass spectrometry

Urinary steroid metabolite profiles were measured using gas chromatography/mass spectrometry as described previously (27). One milliliter of sample was used per assay. Steroid quantification was achieved by comparison to internal standards, and concentrations were expressed as micrograms per 24 h. The urinary ratio of

cortisol metabolites [ratio of tetrahydrocortisol (THF) and 5α-THF to tetrahydrocortisone (THE)] provided an index of global 11β-HSD1 activity (27), although this is believed principally to reflect hepatic activity. Total glucocorticoid metabolites (THF, THE, 5α-THF, α-cortolone, cortisone, cortisol, β-cortolone, β-cortol, and α-cortol) reflected cortisol secretion rate. Ratios of 5α-THF to THF and androsterone (An) to etiocholanolone (Et) infer 5α- and 5β-reductase activities.

Liquid chromatography/mass spectrometry

Total CSF cortisol and cortisone measurements were performed by online solid-phase extraction coupled with liquid chromatography/tandem mass spectrometry (LC/MS). One hundred microliters of CSF were mixed with 20 μl internal standard solution (Cortisol-d4, cortisone-d7; Cambridge Isotope Laboratories, Andover, MA) and diluted with water to reach a final volume of 200 μl. Fifty microliters of sample were injected into the XLC-MS/MS system. Sample cleanup took place by online solid-phase extraction, using a Spark Holland Symbiosis system (Spark Holland, Emmen, The Netherlands) as described previously (28). HySphere C18 HD 10 × 2-mm SPE cartridges (Spark Holland) were used for sample extraction. Chromatographic separation was achieved using an Xbridge C8 (particle size, 3.5 μm; internal diameter, 2.1 by 100 mm; Waters, Milford, MA). Detection was performed with a Quattro Premier tandem mass spectrometer equipped with a Z Spray ion source operated in positive electrospray ionization mode (Waters). Cortisol, cortisone, and their deuterated internal standards were protonated to produce ions at the form $[M^+H]^+$, with m/z 363 and 367 and m/z 361 and 368, respectively. Upon collision-induced dissociation with argon gas, these precursor ions produced characteristic product ions of m/z 121 for both cortisol and its deuterated internal standard. For cortisone and its internal standard, product ions were m/z 163 and 167. The multiple reaction monitoring mode was developed for the specific m/z transitions 363→121 and 367→121 (cortisol and internal standard cortisol) and 361→163 and 368→167 (cortisone and internal standard cortisone). CSF cortisol and cortisone measurements were performed at the University Medical Center Groningen, The Netherlands.

Statistical analysis

Statistical analysis was performed using SPSS version 15 (SPSS Inc., Chicago, IL) and Prism for Windows version 5.0 (GraphPad Software Inc., San Diego, CA). Data were summarized using means and SD values and analyzed by one-way ANOVA (Kruskal-Wallis for nonparametric data). Serial measurements were analyzed using repeated measures ANOVA (for parametric or log-transformed data). Associations between steroid profiles (nonparametric) were analyzed using Spearman correlation. The level at which the results were judged significant was $P < 0.05$. Data for papilledema measures in the right eye only are quoted as both eyes correlated significantly.

Results

Immunohistochemistry

Hematoxylin and eosin staining provided confirmatory histology of CP and AGT (Fig. 2A, i and ii, and B, i and ii, respectively).

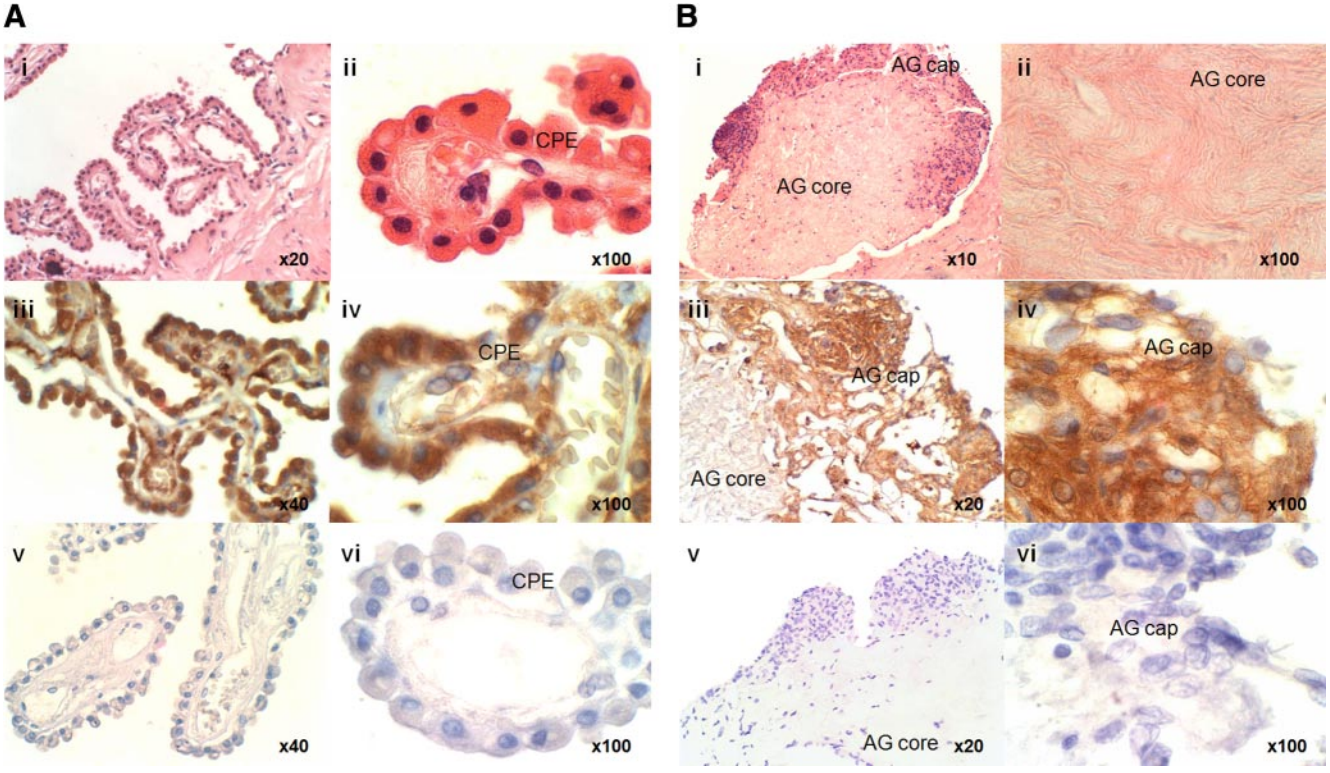

FIG. 2. Hematoxylin and eosin staining provided confirmatory histology of CP (A, i and ii) and AGT (B, i and ii). Immunohistochemistry using antihuman 11β-HSD1 antibody revealed staining in the CP epithelial cells (CPE) (A, iii and iv) and arachnoid granulation cap cell cluster (AG cap; arachnoid granulation epithelial cells that lie over the surface of the arachnoid granulation abutting the lumen of the draining venous sinus) (B, iii and iv). This staining was specifically removed with preadsorbed immunizing peptide (CP, A, v and vi; and AGT, B, v and vi). AG core, Arachnoid granulation core.

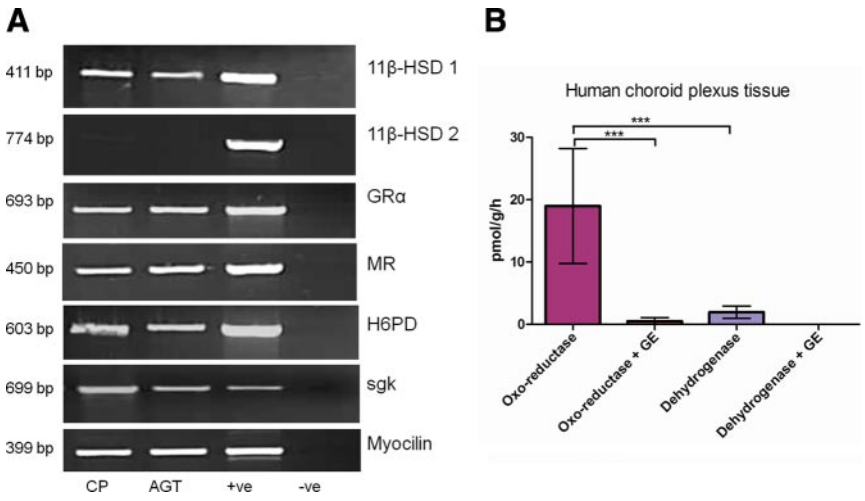

FIG. 3. A, Reverse transcriptase PCR of human CP and AGT demonstrating mRNA expression of 11β-HSD1, GRα, MR, H6PD, sgk-1, and myocilin. 11β-HSD2 was not expressed. Positive control tissue (+ve): human liver for 11β-HSD1, GR, and H6PD; human kidney for sgk and MR; differentiated adipocyte cell line for 11β-HSD2; human heart for myocilin. Negative control (−ve). B, 11β-HSD activity studies in CP tissue (n = 4) demonstrated that oxo-reductase activity was significantly higher than dehydrogenase activity (cortisone to cortisol, 15.5 ± 7.5 pmol/g/h; cortisol to cortisone 2.4 ± 0.6 pmol/g/h). Oxo-reductase activity was significantly inhibited with coincubation with GE. AGT failed to demonstrate 11β-HSD activity. ***, $P < 0.001$.

11β-HSD1 immunoreactivity was confined to the epithelial layer of the CP (Fig. 2A, iii and iv). No staining was observed in sections where the antibody was pretreated with immunizing peptide (Fig. 2A, v and vi). Staining was also seen in the AGT cap cell cluster (Fig. 2B, iii and iv), and specificity for 11β-HSD1 was confirmed via antibody preadsorption with immunizing peptide (Fig. 2B, v and vi). There was no staining for 11β-HSD2 in either CP tissue or AGT. Positive control tissues (liver for 11β-HSD1 and kidney for 11β-HSD2; data not shown) revealed characteristic patterns of staining (24).

mRNA expression

Conventional reverse transcriptase PCR of human CP and AGT tissue identified mRNA expression for key elements of the glucocorticoid signaling pathway [11β-HSD1, GRα, mineralocorticoid receptor (MR), hexose-6-phosphate dehydrogenase (H6PD), sgk, and myocilin]. 11β-HSD2 mRNA was not expressed in either tissue (Fig. 3A).

11β-HSD activity assays

Activity assays conducted on CP tissue explants (n = 4) demonstrated predominant oxo-reductase compared with dehydrogenase activity (cortisone to cortisol, 19.0 ± 9.2 pmol/g/h, *vs.* cortisol to cortisone, 2.0 ± 1.0 pmol/g/h; $P < 0.001$). Oxo-reductase activity was significantly decreased to 0.5 ± 0.6 pmol/g/h by coincubation with the non-specific 11β-HSD1 inhibitor, GE ($P < 0.001$; Fig. 3B). No 11β-HSD activity was detected in the AGT.

Clinical study

Twenty-five subjects were enrolled, and 22 completed the study (one was excluded due to a secondary IIH cause, and two were lost to follow-up). Baseline characteristics are detailed in Table 1. During the observation period (stage 1), weight remained stable (0.8 ± 3.1 kg), and there were no significant changes in any of the outcome measures. The VLCD (stage 2) resulted in significant weight reduction (14.2 ± 7.8 kg; $P < 0.001$), equivalent to a 15.2 ± 7.8% loss of total body weight. BMI decreased by 5.8 ± 3.0 kg/m^2, and waist circumference by 9.8 ± 5.4 cm ($P < 0.001$). As previously reported, ICP, Headache Impact Test-6, and papilledema were also significantly reduced ($P < 0.001$, $P = 0.004$, and $P = 0.002$, respectively) (20).

Urinary steroid metabolites

No changes in the urinary steroid metabolites were noted during stage 1. After the VLCD (stage 2), there was a significant reduction in the THF+5α-THF:THE ratio (from 0.95 ± 0.30 at start of diet to 0.81 ± 0.25 at end of diet; $P = 0.001$) (Fig. 4A and Table 2). The urinary free cortisol:cortisone ratio, an indicator of 11β-HSD2 activity, did not change during the study, suggesting that the change in THF+5α-THF:THE reflected reduced 11β-HSD1 activity. In keeping with reduced 11β-HSD1 activ-

TABLE 1. Demographic changes during study

Characteristic	Baseline	Start of diet	End of diet	*P* value
Weight (kg)	101.3 ± 16.5	101.3 ± 17.4	86.3 ± 15.5	**<0.001**
BMI (kg/m^2)	37.9 ± 4.9	38.1 ± 5.3	32.4 ± 4.9	**<0.001**
Waist (cm)	111.1 ± 10.4	111.1 ± 10.0	101.0 ± 12.0	**<0.001**
ICP (cm CSF)	39.5 ± 4.6	37.8 ± 4.7	29.7 ± 4.7	**<0.001**
Headache Impact Test-6 score	59.2 ± 9.0	54.8 ± 9.3	46.7 ± 10.1	**0.004**
Papilledema, USG optic disc height (mm)	1.02 ± 0.27	0.98 ± 0.29	0.81 ± 0.26	**0.002**

Data are expressed as mean ± SD. No significant changes were noted between baseline and start of diet. *P* values represent changes from start of diet to end of diet. USG, Ultrasonography. *Bold* represents *P* values reaching statistical significance of <0.05.

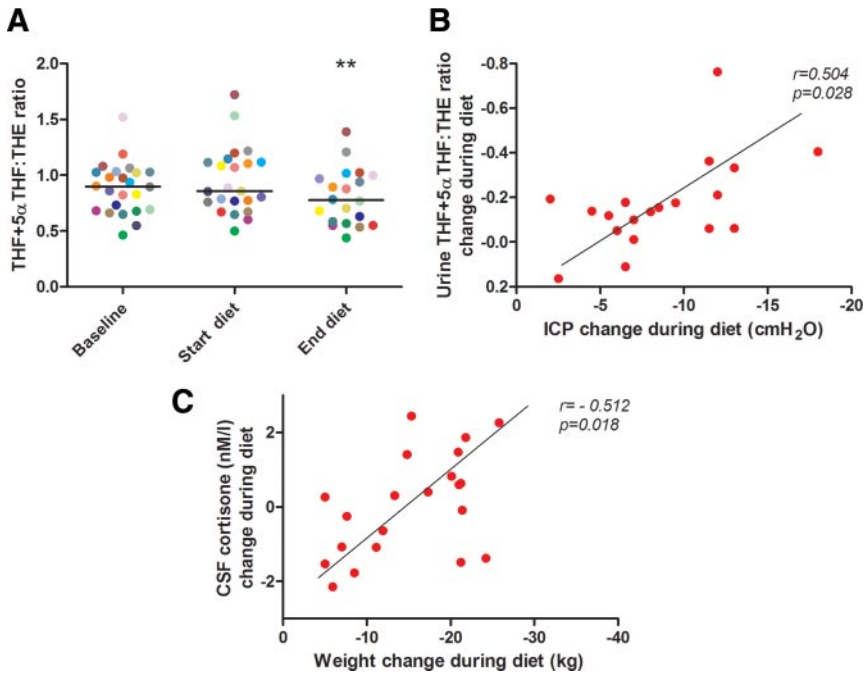

FIG. 4. A, Scatter plot with median and ranges illustrating a significant reduction in urine 11β-HSD 1 activity [THF and 5α-THF to THE ratio] after weight loss. **, $P = 0.001$. B, The change in urine 11β-HSD1 activity correlated with change in ICP after the diet. C, The extent of weight loss inversely correlated with change in CSF cortisone after the diet.

ity, the total cortisol metabolite (Fm) to total cortisone metabolite (Em) ratio also fell (0.73 ± 0.22 *vs.* 0.63 ± 0.19; $P = 0.001$). The change in global 11β-HSD1 activity correlated significantly with the fall in ICP (r = 0.504; $P = 0.028$) (Fig. 4B). Measures of 5α-reductase activity decreased after the VLCD (5α-THF, $P = 0.002$; 5α-THF:THF ratio, $P = 0.005$; An, $P = 0.006$; An:Et ratio, $P = 0.002$; Table 2). Total glucocorticoid metabolites, reflecting daily cortisol secretion rate, also fell after weight loss ($P = 0.013$).

Serum and CSF cortisol and cortisone

Cortisol and cortisone levels in the CSF were lower than that observed in the serum (baseline cortisol, 9.5 ± 5.0 *vs.* 239 ± 116 nM/liter; and cortisone, 5.0 ± 2.0 *vs.* 39.2 nM/liter for CSF and serum, respectively). Cortisol:cortisone ratios were lower in the CSF compared with serum (1.9 ± 0.4 *vs.* 6.0 ± 2.0, respectively), although in the absence of measuring free steroid levels, these ratios should be interpreted with caution. Absolute CSF and serum steroid concentrations did not change after weight loss (Table 2), but the change in CSF cortisone negatively correlated with a change in weight (r = −0.512; $P = 0.018$) (Fig. 4C) and BMI (r = −0.444; $P = 0.044$).

Conclusion

We previously suggested that 11β-HSD1 may influence ICP through the regulation of CSF homeostasis in a fashion analogous to that reported by our group in the ciliary body (22, 23). In keeping with this, we have demonstrated 11β-HSD1 immunoreactivity in the CSF secreting CP epithelial cells. Additionally, key elements of the glucocorticoid signaling pathway were expressed, and 11β-HSD1 enzyme activity was observed. We also demonstrated mRNA expression of 11β-HSD1, GRα, and the glucocorticoid-regulated protein myocilin in AGT. No 11β-HSD1 activity was seen in the AGT. Although this may reflect tissue degeneration resulting from the delay between postmortem and enzyme activity assay (23.6 ± 6 h; range, 16–29 h), bearing in mind the much larger surface area of AGT compared with CP, it is also possible that there could be differences in the posttranslational regulation of 11β-HSD1 activity between CP and AGT. Importantly, we did not examine H6PD activity (a key regulator of 11β-HSD1 directionality) in either CP or AGT. We have, however, confirmed 11β-HSD1 activity in an arachnoid granulation epithelial cell line (Sinclair, A., unpublished data). These findings suggest that both the CP and AGT are potential glucocorticoid target tissues and, whereas the CP has the potential to regulate prereceptor glucocorticoid availability through 11β-HSD1, paracrine actions of cortisol may influence arachnoid granulation function. These results provide a rationale for further investigation of the functional role of glucocorticoids and 11β-HSD1 in CSF secretion and drainage. In contrast to the eye and the measurement of intraocular pressure, no studies have been published showing that inhibition of 11β-HSD1 lowers CSF pressure. The absence of 11β-HSD2 expression combined with the presence of the MR also raises the interesting possibility that altered CSF dynamics could result from glucocorticoid activation of the MR, although this is a hypothesis that remains to be tested. Proinflammatory cytokines including TNFα are potent regulators of 11β-HSD1 activity (29); however, data from our previous studies have shown that CSF TNFα levels are not elevated in IIH (30).

We subsequently characterized glucocorticoid metabolism in IIH subjects before and after weight reduction (loss of 15.2 ± 7.8% of body weight). Global 11β-HSD1 activity decreased as assessed by the urinary THF+5α-THF:THE ratio but 11β-HSD2 activity was unchanged as assessed by the free cortisol:cortisone ratio. The greatest reduction in global 11β-HSD1 activity occurred in IIH

TABLE 2. Steroid metabolite changes during study

Characteristic	Baseline	Start of diet	End of diet	*P* value
Urine (μg/24 h)				
THF+5α-THF:THE	0.89 ± 0.23	0.95 ± 0.30	0.81 ± 0.25	**0.001**
Cortisol:cortisone	0.70 ± 0.19	0.75 ± 0.19	0.69 ± 0.30	0.456
5α-THF:THF	1.18 ± 0.62	1.20 ± 0.64	0.93 ± 0.47	**0.005**
5α-THF	1,883.0 ± 1,502.0	1,868.9 ± 1,370.0	1,031.3 ± 925.3	**0.002**
THF	1,586.8 ± 971.2	1,553.6 ± 659.2	1,053.0 ± 648.5	**0.020**
Total GC metabolites	10,745.1 ± 5,902.6	10,826.2 ± 5,804.5	6,995.4 ± 4,480.2	**0.013**
An	2,424.0 ± 2,121.1	2,579.7 ± 2,273.8	1,536.7 ± 1,609.6	**0.006**
An:Et	1.42 ± 0.69	1.48 ± 0.90	1.16 ± 0.68	**0.002**
An+Et+DHEA	5,361.3 ± 4,849.2	5,352.3 ± 4,920.2	3,679.8 ± 4,004.8	**0.010**
Fm	4,401.7 ± 2,872.9	4,384.9 ± 2,372.3	2,660.1 ± 1,837.3	**0.004**
Em	6,343.5 ± 3,144.6	6,441.3 ± 6,567.0	4,335.3 ± 2,730.9	**0.022**
Fm:Em	0.68 ± 0.14	0.73 ± 0.22	0.63 ± 0.19	**0.001**
CSF (nM/liter)				
Cortisol	9.52 ± 5.00	8.36 ± 3.66	8.60 ± 3.56	0.852
Cortisone	5.04 ± 1.99	4.67 ± 1.69	4.88 ± 1.56	0.944
Cortisol:cortisone ratio	1.85 ± 0.44	1.81 ± 0.37	1.76 ± 0.39	0.619
Serum (nM/liter)				
Cortisol	239.03 ± 115.66	194.48 ± 82.41	190.27 ± 92.63	0.708
Cortisone	39.19 ± 15.78	37.66 ± 15.36	35.39 ± 10.64	0.932
Cortisol:cortisone ratio	6.01 ± 1.95	5.15 ± 1.01	5.15 ± 1.67	0.614
Serum:CSF ratio				
Cortisol	26.33 ± 9.43	24.00 ± 7.49	24.61 ± 11.86	0.754
Cortisone	8.20 ± 3.00	8.23 ± 1.92	7.69 ± 1.43	0.509
Cortisol:cortisone ratio	3.31 ± 0.95	2.94 ± 0.72	3.15 ± 1.24	1.00

Data are expressed as mean ± SD. No significant changes were noted between baseline and start of diet. *P* values represent changes from start of diet to end of diet. GC, Glucocorticoid; An, androsterone; DHEA, dehydroepiandrosterone.

subjects who experienced the largest fall in ICP ($r = 0.04$; $P = 0.028$). Although this observation is consistent with our hypothesis, it does not confirm causality. Previous studies of weight loss in non-IIH obese subjects do not report significant changes in urinary steroid metabolite ratios reflecting global 11β-HSD1 activity (18, 19). However, unpublished subanalysis of females from our idiopathic obesity cohort indicate a trend toward a decrease in 11β-HSD1 activity (J.W.T., personal communication), although the numbers in the idiopathic obesity cohort are much smaller than published in this study, and this may have prevented statically significant changes from being achieved. This comparison would argue against disease-specific regulation in patients with IIH, and it is possible, therefore, that after weight loss there are gender differences in 11β-HSD1 activity. Weight loss may also cause tissue-specific changes in 11β-HSD1, as indicated by elevated adipocyte 11β-HSD1 mRNA expression in obese patients after weight reduction (18, 19).

Reduced 5α-reductase activity, as assessed by the urinary 5α-THF:THF and An:Et ratios, was demonstrated in the IIH subjects after weight loss and has also been noted in obese cohorts (19). The precise role of 5α-reductase (a cortisol-inactivating enzyme), which is highly expressed in liver and adipose tissue, is under investigation (31). Decreased 5α-reductase activity, with consequent reduced inactivation of cortisol, may account for the diminished hypothalamic-pituitary-adrenal axis drive, indicated by the reduced total urinary metabolite excretion noted in the IIH cohort. The observed reduction in 5α-reductase activity is not solely a reflection of cortisol metabolism because 5α-reductase also has a key role in activating testosterone to 5α-dihydrotestosterone (32). Changes in androgen metabolism may have therapeutic implications in the female-biased condition of IIH. Interestingly, both 5α-reductase and 11β-HSD1 are regulated by estrogen, with higher levels demonstrated in females (33, 34). The role of 5α-reductase activity in IIH patients warrants further exploration.

In an attempt to further analyze the relevance of 11β-HSD1 expression in the CP, we carried out the novel quantification of both cortisol and cortisone in CSF using LC/MS. CSF cortisol and cortisone levels were observed to be much lower in the CSF than serum, with concentrations mirroring those observed in aqueous humor (22), although much lower than that demonstrated in other fluids such as synovial fluid (35). These results pose many questions, not the least of which is the impact of cortisol binding globulin on free glucocorticoid levels in the CSF. Previous studies report variable levels of protein-bound cortisol within the CSF, although free CSF cortisol levels typically mirror those observed in the serum (36–38). CSF cortisone concentrations have, to our knowledge, not been previously evaluated. Because serum cortisone has

limited binding to cortisol binding globulin, and thus total levels are a close approximation to free cortisone levels (39), equilibration across the blood-brain barrier would be predicted. However, CSF levels are notably lower than those measured in serum (serum CSF cortisone ratio, 8.2), suggesting local conversion of cortisone to cortisol by 11β-HSD1. Neither the CSF nor serum cortisol or cortisone levels significantly changed with weight loss. However, it is likely that this approach to measuring *in vivo* changes in CP 11β-HSD1 activity is too simplistic because CSF glucocorticoids will reflect local tissue steroid metabolism as well as glucocorticoid transfer across the blood-brain barrier. The precise contribution of CP 11β-HSD1 to CSF cortisol and cortisone concentrations is unresolved. The lack of significant change in serum glucocorticoid levels mirrors findings in non-IIH obese subjects and is in keeping with decreased cortisol secretion (mediated by reduced 11β-HSD1) offset by increased adrenal cortisol secretion driven by the hypothalamic-pituitary-adrenal axis (19). Weight loss correlated with CSF cortisone levels ($r = -0.512$; $P = 0.018$) in that those individuals who lost the most weight had the greatest change in CSF cortisone. Although we did not see changes in CSF cortisol, these observations may reflect a reduction in 11β-HSD1 activity.

We acknowledge that a stress effect of the VLCD may have impacted on our results. A single serum cortisol measurement before and after weight loss is not sufficient to draw any firm conclusions, but these did not differ significantly. More frequent sampling may have provided more robust data. Further studies using different dietary interventions are now warranted, not only to determine their clinical impact in IIH, but also to accurately define diet-specific impact upon glucocorticoid metabolism. The duration, magnitude, and macronutrient composition of the calorie restriction are all potentially important (40), but as yet untested in patients with IIH. We also accept that the benefits of therapeutic weight loss in IIH may not relate exclusively to 11β-HSD1 because changes in adipose tissue, an active endocrine organ, with subsequent alterations on the inflammatory cytokine and adipokine profiles, particularly leptin (30), may have impacted on CSF dynamics.

In summary, we describe decreased 11β-HSD1 activity with weight loss, which correlates with improved ICP in IIH patients. The possible involvement of 11β-HSD1 in IIH and ICP homeostasis is further suggested through the expression of 11β-HSD1 and the glucocorticoid signaling pathway in CP and AGT. Elevated 11β-HSD1 may represent a pathogenic mechanism in IIH, and inhibition of 11β-HSD1 may represent a therapeutic strategy for the treatment of IIH. Additionally, our use of LC/MS to analyze both CSF cortisol and cortisone provides a novel methodology for future CSF glucocorticoid studies.

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This work was supported by the Vera Down (Neurological Diseases) 2005 and Clark & McMaster (Blinding Diseases) 2005 Awards, British Medical Association, United Kingdom; a Research and Development 2005 award from Sandwell and West Birmingham Hospitals National Health Service Trust, and by The West Midlands Neuroscience Teaching and Research Fund, United Kingdom. A.J.S. is a Brain Trust Entry/Exit Scholar (Institute of Neurology, London, UK), Ipsen Research Fellow (London, UK), and Medical Research Council Clinical Training Fellow (London, UK). The Academic Unit of Ophthalmology is supported by the Birmingham Eye Foundation (Registered UK Charity 257549). J.W.T. is a MRC Senior Clinical Fellow. The Parkinson's Disease Society Tissue Bank at Imperial College (London, UK) is funded by the Parkinson's Disease Society of the United Kingdom (Registered Charity 948776).

Disclosure Summary: The authors have nothing to disclose. Lipotrim, Howard Foundation, Cambridge, UK, had no role in funding this study or in the study design, data collection, analysis, data interpretation, report writing, or the decision to submit the manuscript.

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