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RESEARCH ARTICLE

Identification of a human blood biomarker of pharmacological 11β-hydroxysteroid dehydrogenase 1 inhibition

Cristina Gómez[1] | Zerin Alimajstorovic[2] | Nantia Othonos[3] | Denise V. Winter[1] | Sarah White[3] | Gareth G. Lavery[4] | Jeremy W. Tomlinson[3] | Alexandra J. Sinclair[2,5] | Alex Odermatt[1]

[1]Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland

[2]Metabolic Neurology, Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK

[3]Oxford Centre for Diabetes, Endocrinology and Metabolism, NIHR Oxford Biomedical Research Centre, University of Oxford, Churchill Hospital, Oxford, UK

[4]Department for Biosciences, Nottingham Trent University, Nottingham, UK

[5]Department of Neurology, University Hospitals Birmingham, Birmingham, UK

Correspondence
Alex Odermatt, Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland.
Email: alex.odermatt@unibas.ch

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Abstract

Background and Purpose: 11β-Hydroxysteroid dehydrogenase-1 (11β-HSD1) catalyses the oxoreduction of cortisone to cortisol, amplifying levels of active glucocorticoids. It is a pharmaceutical target in metabolic disease and cognitive impairments. 11β-HSD1 also converts some 7oxo-steroids to their 7β-hydroxy forms. A recent study in mice described the ratio of tauroursodeoxycholic acid (TUDCA)/tauro-7oxolithocholic acid (T7oxoLCA) as a biomarker for decreased 11β-HSD1 activity. The present study evaluates the equivalent bile acid ratio of glycoursodeoxycholic acid (GUDCA)/glyco-7oxolithocholic acid (G7oxoLCA) as a biomarker for pharmacological 11β-HSD1 inhibition in humans and compares it with the currently applied urinary (5α-tetrahydrocortisol + tetrahydrocortisol)/tetrahydrocortisone ((5αTHF + THF)/THE) ratio.

Experimental Approach: Bile acid profiles were analysed by ultra-HPLC tandem-MS in blood samples from two independent, double-blind placebo-controlled clinical studies of the orally administered selective 11β-HSD1 inhibitor AZD4017. The blood GUDCA/G7oxoLCA ratio was compared with the urinary tetrahydro-glucocorticoid ratio for ability to detect 11β-HSD1 inhibition.

Key Results: No significant alterations were observed in bile acid profiles following 11β-HSD1 inhibition by AZD4017, except for an increase of the secondary bile acid G7oxoLCA. The enzyme product/substrate ratio GUDCA/G7oxoLCA was found to be more reliable to detect 11β-HSD1 inhibition than the absolute G7oxoLCA concentration in both cohorts. Comparison of the blood GUDCA/G7oxoLCA ratio with the urinary (5αTHF + THF)/THE ratio revealed that both successfully detect 11β-HSD1 inhibition.

Conclusions and Implications: 11β-HSD1 inhibition does not cause major alterations in bile acid homeostasis. The GUDCA/G7oxoLCA ratio represents the first blood

Abbreviations: GUDCA, glycine conjugated UDCA; LOQ, limit of quantification; 7oxoLCA, 7oxolithocholic acid; THE, tetrahydrocortisone; αTHF, 5α-tetrahydrocortisol; THF, 5β-tetrahydrocortisol; TUDCA, taurine conjugated UDCA.

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biomarker of pharmacological 11β-HSD1 inhibition and may replace or complement the urinary (5αTHF + THF)/THE ratio biomarker.

KEYWORDS

11β-hydroxysteroid dehydrogenase, bile acid, biomarker, disease, glucocorticoid, inhibitor, LC–MS

1 | INTRODUCTION

Glucocorticoids belong to the most widely prescribed drugs, and are used to treat chronic asthma and different inflammatory conditions or as co-medication following organ transplantation (Cain & Cidlowski, 2017; Reichardt et al., 2021). However, the long-term pharmacological use of glucocorticoids or chronically elevated endogenous glucocorticoid levels promote various adverse health effects including cardio-metabolic complications, osteoporosis, glaucoma and skeletal muscle atrophy as well as depression and cognitive impairment. Glucocorticoids exert their functions mainly by activating **glucocorticoid receptors** (GR). At a tissue- and cell-specific level, the concentrations of active glucocorticoids are tightly controlled by **11β-hydroxysteroid dehydrogenase** (11β-HSD) enzymes (Gathercole et al., 2013; Odermatt & Kratschmar, 2012). 11β-HSD1 is widely expressed, and, in metabolically active tissues such as liver, adipose, bone and skeletal muscle, it converts the inactive glucocorticoid cortisone to the active form cortisol (Figure 1). Inappropriately elevated levels of 11β-HSD1 activity have been implicated in many diseases including cardio-metabolic disorders, impaired wound healing and cognitive impairment; thus, inhibition of 11β-HSD1 represents an attractive therapeutic strategy to lower glucocorticoid-mediated adverse effects (Gathercole et al., 2013; Gregory et al., 2020; Scott et al., 2014). Therefore, a variety of pharmacological 11β-HSD1 inhibitors have been developed and tested in clinical trials (Bianzano et al., 2021; Courtney et al., 2008; Feig et al., 2011; Freude et al., 2016; Hardy et al., 2021; Heise et al., 2014; Markey et al., 2020; Othonos et al., 2023; Schwab et al., 2017; Shah et al., 2011; Webster et al., 2017).

Despite promising preclinical data, the translation to clinical application has been challenging. To better monitor pharmacological 11β-HSD1 inhibition in humans, the identification of novel non-invasive biomarkers reporting 11β-HSD1 inhibition could greatly facilitate optimization of treatment regimens. Current measurements to assess systemic 11β-HSD1 activity include *i.v.* injection of prednisone followed by determination of formed prednisolone in blood samples (Bhat et al., 2008; Courtney et al., 2008), thus requiring an additional time-consuming and expensive intervention. Alternatively, systemic 11β-HSD1 activity in humans can be assessed by determining the ratios of the A-ring 5α- and 5β-reduced metabolites of cortisol and cortisone, that is, the (5α-tetrahydrocortisol [αTHF] + 5β-tetrahydrocortisol [THF])/tetrahydrocortisone (THE) ratio and the THF/THE ratio in 24-h urine samples (Bianzano et al., 2021; Courtney et al., 2008; Freude et al., 2016; Jamieson et al., 1999; Markey et al., 2020; Sagmeister et al., 2019; Tomlinson & Stewart, 2001; Webster et al., 2017). This ratio is also influenced by 11β-HSD2 that converts cortisol to cortisone, mainly in the kidney and the colon, exemplified by the highly elevated (αTHF + THF)/THE ratio in urines from patients with genetic defects in 11β-HSD2 (Odermatt et al., 2001; Palermo et al., 1996; Shackleton, 1993). Obtaining complete and accurate 24-h urine samples is difficult, has poor patient acceptability and leads to challenges in sample storage and further processing. Currently, a suitable blood biomarker to report 11β-HSD1 inhibition is lacking.

11β-HSD1 is a multi-functional enzyme that in the presence of hexose-6-phosphate dehydrogenase (H6PD) predominantly functions as an oxoreductase (Odermatt & Klusonova, 2015). Earlier studies showed that human 11β-HSD1 is capable of metabolizing

What is already known

- The glucocorticoid metabolizing enzyme 11β-HSD1 can also reduce the secondary bile acid 7-oxolithocholic acid.
- The TUDCA/T7oxoLCA ratio detects decreased 11β-HSD1 activity in transgenic mice and upon pharmacological inhibition.

What does this study add

- The blood GUDCA/G7oxoLCA ratio serves as biomarker of pharmacological 11β-HSD1 inhibition in humans.
- This study translates findings from preclinical investigations in mice to humans.

What is the clinical significance

- The blood GUDCA/G7oxoLCA ratio is a biomarker of pharmacological 11β-HSD1 inhibition in humans.
- Blood is easier accessible than 24-h urines; GUDCA/G7oxoLCA could replace (5αTHF + THF)/THE as biomarker.

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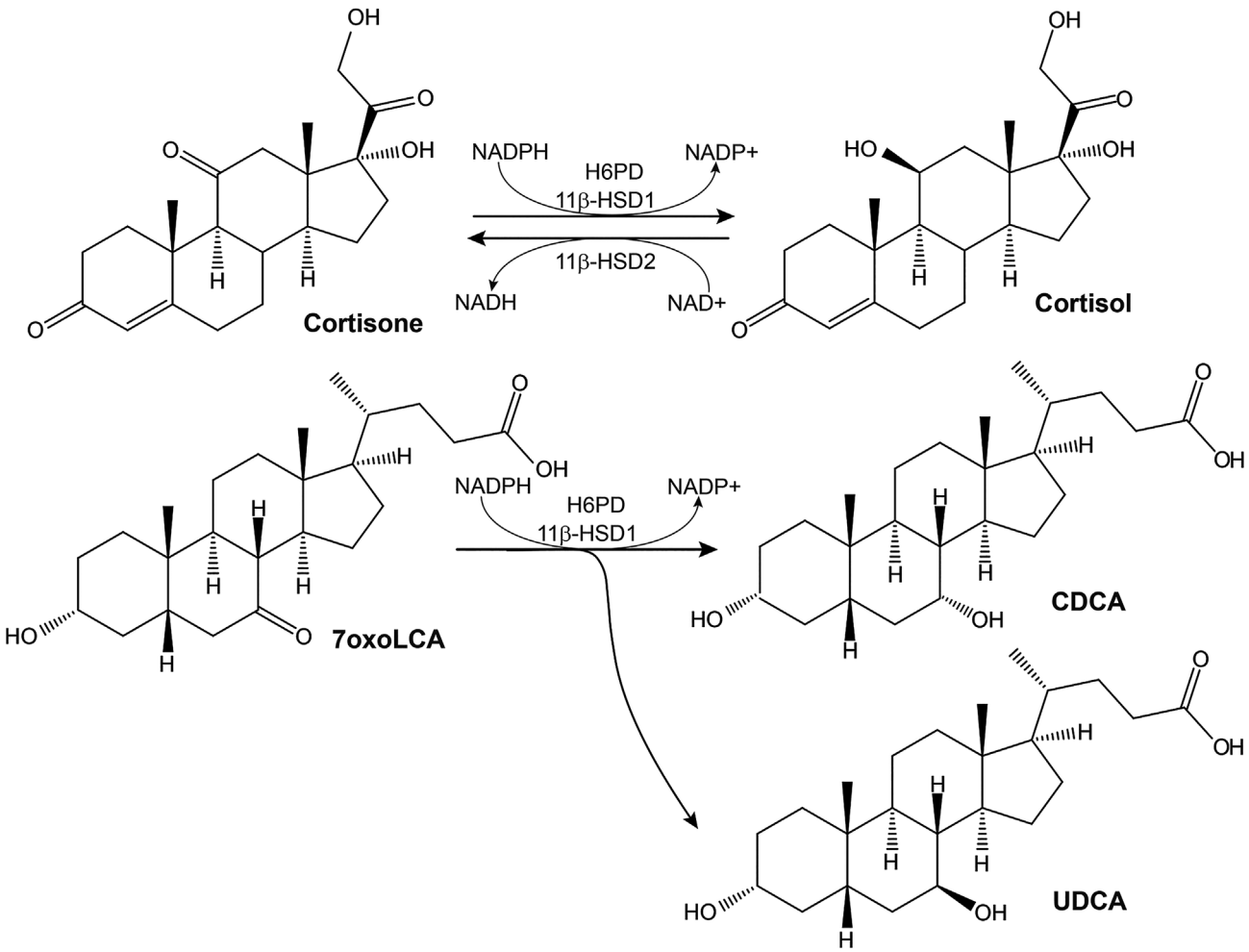

FIGURE 1 Scheme of 11β-hydroxysteroid dehydrogenase (11β-HSD) activity. 11β-HSD1 in the presence of hexose-6-phosphate dehydrogenase (H6PD) catalyses the oxoreduction of cortisone to cortisol and 7oxo-lithocholic acid (7oxoLCA) to ursodeoxycholic acid (UDCA) and lower amounts of chenodeoxycholic acid (CDCA). 11β-HSD2 catalyses the oxidation of cortisol to cortisone but does not accept bile acids as substrates.

7-oxo-cholesterol metabolites (Beck, Inderbinen, et al., 2019; Beck, Kanagaratnam, et al., 2019; Hult et al., 2004; Mitić et al., 2013; Schweizer et al., 2004) but also the secondary bile acid 7oxolithocholic acid (7oxoLCA) to **ursodeoxycholic acid** (UDCA) and to a lesser extent to **chenodeoxycholic acid** (CDCA) (Figure 1) (Mitić et al., 2013; Odermatt et al., 2011; Penno et al., 2013). A lack of this activity in guinea-pigs and in 11β-HSD1-deficient mice results in an accumulation of 7oxoLCA and its taurine conjugated form (Penno et al., 2013, 2014; Weingartner et al., 2021). A recent preclinical study employing three different transgenic mouse models of 11β-HSD1 deficiency and a model of pharmacological inhibition proposed the ratio of taurine conjugated UDCA (TUDCA) to 7oxoLCA (T7oxoLCA) in plasma samples as a biomarker to detect decreased 11β-HSD1 oxoreductase activity (Weingartner et al., 2021). The TUDCA/T7oxoLCA ratio was significantly lower in plasma from 11β-HSD1 knockout (KO) and hexose-6-phosphate dehydrogenase (H6PD) KO mice and in plasma of mice treated with the inhibitor **carbenoxolone**.

The present study aimed to translate the observations made in these mouse models to humans and identify an equivalent blood biomarker reporting decreased 11β-HSD1 activity following pharmacological inhibition. Bile acids are mainly conjugated with taurine in mice but in humans with glycine and to a lesser extent taurine, with an approximate ratio of 3:1 in human adult males (Russell & Setchell, 1992). Therefore, the ratio of glycine conjugated UDCA (GUDCA) to 7oxoLCA (G7oxoLCA) was tested. A liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was applied to assess the plasma or serum bile acid profiles in two different double-blind placebo-controlled clinical studies: a cohort of healthy males cotreated with **prednisolone** and a cohort of females with intracranial hypertension (Hardy et al., 2021; Markey et al., 2020; Othonos et al., 2023). Both groups were orally administered with the selective 11β-HSD1 inhibitor **AZD4017** (Scott et al., 2012). The effects of the inhibitor on the bile acid profile and the capability of the GUDCA/G7oxoLCA ratio to detect 11β-HSD1 inhibition were assessed.

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2 | METHODS

2.1 | Chemicals and reagents

Ultrapure water was obtained using a Milli-Q® Integral purification system equipped with an EDS-Pak® Endfilter for the removal of endocrine active substances (Merck Millipore, Burlington, MA, USA). Acetonitrile (HPLC-S Grade) was purchased from Biosolve (Dieuze, France), methanol (CHROMASOLV™ LC–MS grade) from Honeywell (Charlotte, NC, USA), isopropanol (EMSURE® for analysis) from Merck Millipore and formic acid (Puriss. p.a. ≥ 98%) from Sigma-Aldrich (St. Louis, MO, USA). Bile acids and internal standards were purchased from Sigma-Aldrich or Steraloids (Newport, RI, USA) (Gómez et al., 2020). 11β-HSD1 inhibitor AZD4017 was obtained from AstraZeneca (Cambridge, UK) (Scott et al., 2012).

2.2 | Clinical cohorts

2.2.1 | Cohort A

Healthy male volunteers without diabetes (n = 29, BMI 20–30 $kg \cdot m^{-2}$, 18–60 years) were randomized in a double-blind placebo-controlled study to determine if co-administration of the selective 11β-HSD1 inhibitor AZD4017 limits the adverse effects of short-course exogenous glucocorticoid administration. All volunteers received 20 mg prednisolone once daily and 400 mg AZD4017 or placebo twice daily for 7 days. Blood samples were collected in the morning at baseline, and after 7 days of treatment, and serum was prepared and stored at −80°C until analysis. The trial was registered at Clinicaltrials.gov (NCT03111810, targeting iatrogenic Cushing's syndrome with 11β-hydroxysteroid dehydrogenase Type 1 inhibition [TICSI]) (Othonos et al., 2023). The clinical parameters used for the calculation of correlations with the measured bile acids and ratios thereof are summarized in Table S1.

2.2.2 | Cohort B

Female patients (n = 29, 18–55 years, BMI 25–52 $kg \cdot m^{-2}$) with a clinical diagnosis of active idiopathic intracranial hypertension (IIH) (intracranial pressure [ICP] > 25 cmH_2O and active papilledema) and normal brain imaging were randomized in a double-blind placebo-controlled study (Markey et al., 2017, 2020). Participants received twice daily for 12 weeks 400 mg of the oral selective 11β-HSD1 inhibitor AZD4017 or placebo. Morning blood samples were collected at baseline and following 12 weeks of treatment, and plasma was prepared by centrifugation at 4°C for 10 min at 1500 × *g*, aliquoted and stored at −80°C until analysis. The trial was registered at Clinicaltrials.gov (NCT02017444) and European Clinical Trials Database (EudraCT Number: 2013-003643-31) (Markey et al., 2017). The clinical parameters used for the calculation of correlations with the measured bile acids and ratios thereof are summarized in Table S2 (details of their ascertainment have been previously published) (Hardy et al., 2021; Markey et al., 2017).

2.3 | Sample preparation

Sample material (plasma and serum) from both cohorts was extracted, measured and analysed in a blinded and simple randomized design. Final sample batches from LC–MS analysis were transferred to the Microsoft Excel programme and unblinded for statistical evaluations. For the analysis of bile acids, 25 µl of serum (cohort A) or plasma (cohort B) was diluted 1:4 (v/v) with Milli-Q water. Samples were subjected to protein precipitation by adding 1 ml of 2-propanol containing a mixture of deuterated internal standards. The extraction was performed by continuous shaking for 30 min at 4°C at 10 × g on a Thermomixer C (Eppendorf AG, Hamburg, Germany) and then centrifuged at 16,000 × *g* for 10 min. Supernatants were transferred to new tubes and evaporated to dryness by using a Genevac EZ-2 system (SP Scientific, Warminster, PA, USA) at 35°C. The extracts were reconstituted with 100 µl of methanol to water (1:1, v/v), incubated at 4°C for 10 min at 10 × g, sonicated in a water bath for 10 min at room temperature and finally transferred to LC–MS vials for analysis (Gómez et al., 2020).

2.4 | LC–MS/MS analysis

Samples were analysed by LC–MS/MS, consisting of an Agilent 1290 UPLC coupled to an Agilent 6490 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Agilent Technologies, Basel, Switzerland). The drying gas as well as nebulizing gas was nitrogen. The desolvation gas flow was set at 15 $L \cdot min^{-1}$, the sheath gas flow at 11 $L \cdot min^{-1}$ and the nebulizer pressure at 20 psi. The nitrogen desolvation temperature was set at 290°C and sheath gas temperature at 250°C. Capillary voltage was optimized for each segment from 2000 to 5000 V. Nozzle voltage was set at 2000 V and cell accelerator voltage at 5 V. Chromatographic separation of the bile acids was achieved using reversed-phase column (ACQUITY UPLC BEH C18, 1.7 mm, 2.1 µm, 150 mm, Waters, Wexford, Ireland). The flow rate was set at 0.5 $ml \cdot min^{-1}$ and the column temperature at 55°C. The mobile phase consisted of ultrapure water and acetonitrile (95:5, v/v) with 0.1% formic acid (solvent A) and acetonitrile and ultrapure water (95:5, v/v) with 0.1% formic acid (solvent B) (Gómez et al., 2020).

2.4.1 | Bile acid profile

The following gradient pattern was used for the separation of bile acids: 0 min, 25% B; 3.1 min, 35% B; 9 min, 38% B; 15 min, 65% B; 18 min, 65% B; 20 min, 100% B; 22 min, 25% B; and additional 2 min post-run at initial conditions. The injection volume was set at 3 µl.

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Data acquisition was performed using multiple reaction monitoring (MRM) mode. At least two transitions (quantifier and qualifier transitions) were selected for each compound in positive or negative ESI mode depending on the compound. Collision energy was optimized for each transition as described earlier (Gómez et al., 2020).

2.4.2 | Bile acid ratio-specific method

The separation of GUDCA, GCDCA and G7oxoLCA was achieved with the following gradient pattern: 0 min, 35% B; 4 min, 55% B; 5.50 min, 100% B; for 1 min, 100% B; and additional 1.5 min post-run at initial conditions. The injection volume was set at 5 µl. Data acquisition was performed using MRM mode.

2.4.3 | Quantification of AZD4017

For the analysis of the 11β-HSD1 inhibitor AZD4017, the gradient applied was as follows: 0 min, 45% B; 5 min, 90% B; 5.5 min, 100% B; 9 min, 100% B; 9.5 min, 45% B. The injection volume was set at 2 µl. Data acquisition was performed in ESI positive mode with the following MRM transitions: *m/z* 420.2 to 321.1 (CE 29 V) and *m/z* 420.2 to 279 (CE 37 V).

2.5 | Data analysis and statistics

For LC–MS/MS data, MassHunter Acquisition Software and Quantitative Analysis vB.07.01 (Agilent Technologies, Inc., Santa Clara, CA, USA) were used for quantification. The non-parametric Kruskal–Wallis test and Dunn's multiple comparison were used to analyse significance of differences between groups. Spearman rank correlation was used to evaluate correlation between different variables. Grubbs' test was performed to determine outliers. Independent of statistic validity no outliers were excluded. Statistical significance was established at $P < 0.01$. Statistical analysis and graphs were performed using GraphPad Prism v5.02 (GraphPad Software, Inc., San Diego, CA, USA) and RStudio v1.4.1106 (RStudio, PBC, Boston, MA, USA). The data and statistical analysis comply with the recommendations on experimental design and analysis of the *British Journal of Pharmacology* (Curtis et al., 2022).

2.6 | Unequal sample size

The present study re-analysed plasma and serum samples from previously published clinical studies (Hardy et al., 2021; Othonos et al., 2023). Unequal sample sizes within the present study are thus related to unequal group numbers within the original study (Cohort B) (Hardy et al., 2021). Additionally, blinded samples for both cohorts were controlled for sufficient volume prior to analysis of bile acid profiles by LC–MS/MS. Both samples of pre- and post-administration for a given study subject were excluded from the analysis if the volume of one of the two samples was insufficient. Excluded samples were attributed to the placebo or AZD4017 administration group during the unblinding process of all measured samples. Cohort A: Exclusion of one subject pre- and post-administration of placebo. Cohort B: two subjects pre- and post-administration of placebo.

2.7 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Cidlowski, et al., 2021; Alexander, Fabbro, et al., 2021).

3 | RESULTS

3.1 | Impact of pharmacological 11β-HSD1 inhibition on serum bile acid profiles assessed in healthy males (cohort A)

Loss of 11β-HSD1 activity in transgenic mice leads to increased serum bile acid levels (Weingartner et al., 2021). We therefore first aimed to assess the effects of 11β-HSD1 inhibition by AZD4017 on serum bile acid profiles in healthy male volunteers. This cohort A consisted of 29 healthy male volunteers, distributed randomly into placebo and AZD4017 treatment groups (Othonos et al., 2023). All participants were simultaneously treated with prednisolone. To cover a broad range of bile acids, a recently developed LC–MS/MS-based method (Gómez et al., 2020) was applied to quantify a series of unconjugated and conjugated bile acids in serum samples at baseline and Post-Administration for both placebo and AZD4017 treatment groups. The mean serum concentrations of major individual bile acids, the sums of unconjugated and conjugated and the sum of all measured bile acids are shown in Table 1. No effects due to prednisolone treatment were observed. Furthermore, no significant changes were detected in the levels of the major bile acids analysed following AZD4017 treatment. A trend decrease was observed for CA, CDCA and UDCA upon 11β-HSD1 inhibition. G7oxoLCA was below the limit of quantification (LOQ) of the method (Gómez et al., 2020) in most of the samples analysed from the baseline and placebo groups (Table S7). However, upon 11β-HSD1 inhibition, G7oxoLCA levels increased at least 10-fold. To estimate the GCDCA/G7oxoLCA and GUDCA/G7oxoLCA ratios, a value of LOQ/2 was assigned to samples that could not be quantified. The calculated ratios were significantly lower in the AZD4017 Post-Administration group compared to the other three groups (Table 1), reporting the 11β-HSD1 inhibition.

To achieve a higher sensitivity for the quantification of G7oxoLCA, allowing calculation of the GCDCA/G7oxoLCA and GUDCA/G7oxoLCA ratios, a second LC–MS/MS method was developed (see Section 2.4.2). This method showed improved analytical

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TABLE 1 Concentrations of individual bile acids in human serum from cohort A.

Cohort A	Placebo Baseline (n = 14) nmol·L^{-1} (mean ± SD)	AZD4017 Baseline (n = 15) nmol·L^{-1} (mean ± SD)	Placebo Post-Administration (n = 14) nmol·L^{-1} (mean ± SD)	AZD4017 Post-Administration (n = 15) nmol·L^{-1} (mean ± SD)
Unconjugated				
CA	458 ± 623	465 ± 1230	393 ± 826	181 ± 216
CDCA	420 ± 467	529 ± 1301	450 ± 770	163 ± 127
DCA	459 ± 440	761 ± 1641	482 ± 683	444 ± 332
UDCA	129 ± 220[a]	64 ± 101[a]	161 ± 391[a]	30 ± 30
LCA	13.4 ± 7.8	13.2 ± 7.9	12.6 ± 5.0	15.6 ± 8.7
7oxoLCA	11.4 ± 11.2[a]	6.8 ± 7.1[a]	9.9 ± 12.5[a]	5.2 ± 3.8[a]
12oxoLCA	9.7 ± 7.0[a]	24.3 ± 50.9[a]	13.2 ± 22.3[a]	21.9 ± 41.8[a]
Conjugated				
GCA	218 ± 234	127 ± 90	274 ± 239	261 ± 226
GCDCA	854 ± 866	369 ± 230	944 ± 957	808 ± 620
GDCA	363 ± 560	214 ± 207	323 ± 302	578 ± 571
GUDCA	228 ± 475	52.2 ± 62.7	163 ± 258	88.8 ± 81.7
G7oxoLCA	0.4 ± 0.6[a]	0.2 ± 0[a]	0.5 ± 0.6[a]	4.1 ± 3.7[*,†,‡]
GLCA	14.7 ± 12.7[a]	11.9 ± 12.7	13.2 ± 13.7[a]	23.9 ± 24.2
TCA	20.7 ± 26.1[a]	11.3 ± 14.7[a]	18.3 ± 28.5[a]	26.3 ± 27.1
TCDCA	81.7 ± 97.4	40.7 ± 25.3	83 ± 175	83 ± 82
TDCA	27.8 ± 41.5	19.2 ± 16.4	14.0 ± 9.6	43.0 ± 45.9
TLCA	2.1 ± 2.2[a]	1.2 ± 1.0[a]	1.3 ± 1.1[a]	2.6 ± 2.3[a]
Total unconjugated	1500 ± 1502	1864 ± 4238	1521 ± 2424	861 ± 606
Total conjugated	1810 ± 2167	847 ± 542	1834 ± 1557	1919 ± 1548
Total bile acids	3311 ± 3369	2711 ± 4374	3355 ± 3396	2780 ± 1900
Ratios				
GCDCA/G7oxoLCA	3276 ± 3019	2002 ± 1315	2735 ± 1526	241 ± 98[*,†,‡]
GUDCA/G7oxoLCA	645 ± 782	284 ± 353	400 ± 267	24 ± 13[*,†,‡]

Note: Unequal samples sizes are the result of the exclusion of one subject pre- and post-administration of placebo due to limited sample amount. Bile acids were quantified by LC-MS/MS. The GUDCA/G7oxoLCA and GCDCA/G7oxoLCA ratios were assessed as potential biomarkers of 11β-HSD1 inhibition. Values are expressed as mean ± SD (nmol·L^{-1}).

[a]Not all of the values were within the limit of quantification (LOQ); the value LOQ/2 was assigned for samples that were below LOQ. No outliers were excluded.

*P < 0.01 for Placebo Baseline versus AZD4017 Post-Administration groups.†P < 0.01 for AZD4017 Baseline versus AZD4017 Post-Administration groups.

‡P < 0.01 for Placebo Post-Administration versus AZD4017 Post-Administration groups.

sensitivity by including only the three bile acids needed for the ratios as well as increasing dwell times and injection volume, allowing for a shorter time-frame for analysis. All previously extracted samples were reanalysed using this short and focused method, and G7oxoLCA could be successfully quantified in all samples. The mean concentrations of the three bile acids and of the GCDCA/G7oxoLCA and GUDCA/G7oxoLCA ratios are shown in Table S3. G7oxoLCA levels were significantly increased in the AZD4017 Post-Administration group when compared to the other three groups. Furthermore, the GCDCA/G7oxoLCA and GUDCA/G7oxoLCA ratios were both significantly decreased in the AZD4017 Post-Administration group compared to the other groups (Figure 2a,b and Table S3).

Several clinical parameters determined in serum and overnight urine samples were collected from the participants (Table S1). Serum lipids (cholesterol, high-density lipoprotein [HDL] and triglycerides) and markers of liver function (bilirubin, albumin, gamma-glutamyl transferase [GGT], alanine aminotransferase [ALT], aspartate aminotransferase [AST] and alkaline phosphatase [ALP]) were not different between the four groups, and ADZ4017 treatment did not affect these parameters. Also, the serum levels of the gonadal steroid testosterone and of the sex hormone binding globulin (SHBG) did not differ among the four groups. In contrast, the serum concentrations of the adrenal androgens androstenedione and DHEAS and of the glucocorticoid cortisol as well as that of the urinary glucocorticoid metabolites

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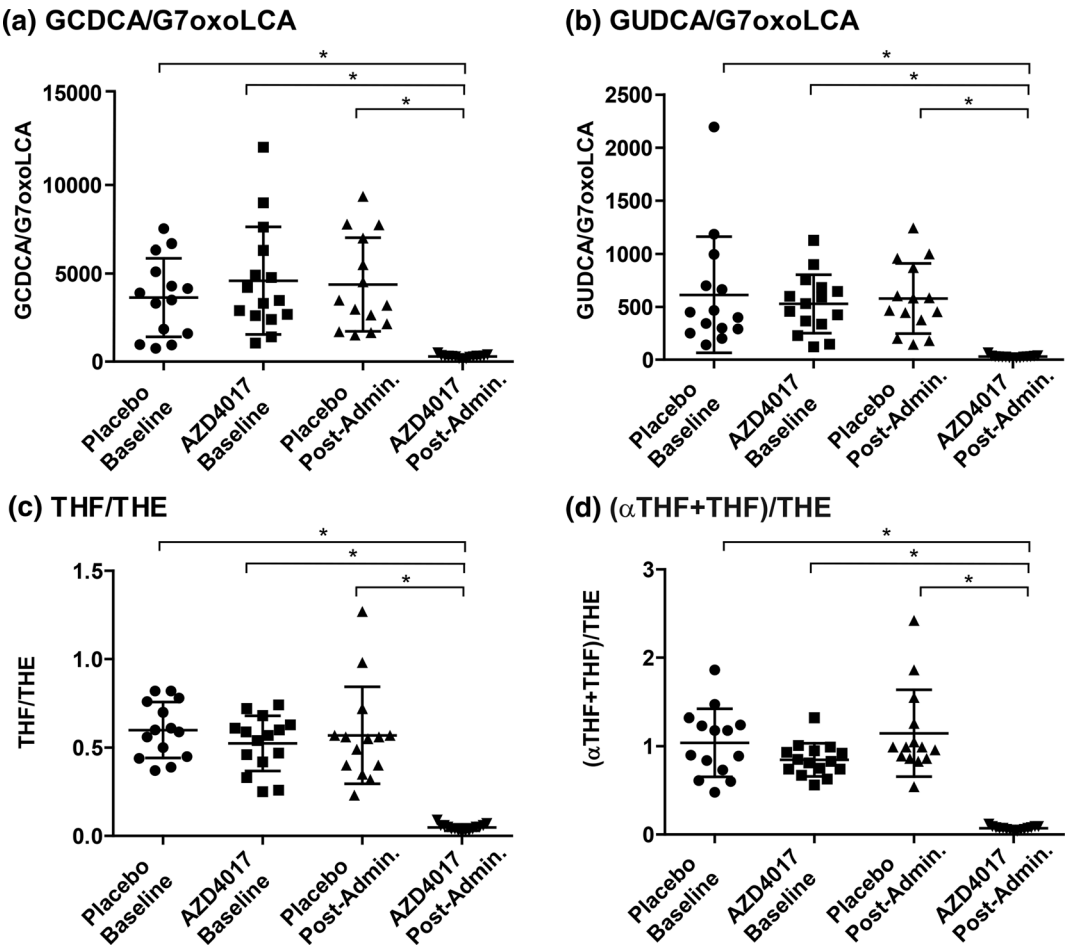

FIGURE 2 Serum bile acid ratios and urine glucocorticoid metabolite ratios as biomarkers of 11β-HSD1 inhibition. The ratios GCDCA/G7oxoLCA (a) and GUDCA/G7oxoLCA (b) in human serum from cohort A were determined by quantification of the respective bile acids by LC–MS/MS. The ratios THF/THE (c) and (αTHF + THF)/THE (d) in overnight urine samples from cohort A were determined by GC–MS as reported earlier (Othonos et al., 2023). Values are expressed as mean ± SD. *$P \leq 0.01$. No outliers were excluded.

decreased significantly in the placebo Post-Administration group compared to the two baseline groups. It needs to be noted that both placebo and AZD4017 treatment groups received prednisolone, which suppresses adrenal steroidogenesis. This is supported by the lower serum levels of ACTH in the placebo Post-Administration group. AZD4017 treatment reversed the decreased serum **ACTH** and adrenal androgen levels as well as those of cortisol and cortisone in 24-h urine samples. Importantly, while the 11β-HSD2 activity marker, that is, the urinary cortisol/cortisone ratio, was not altered by AZD4017 treatment, both urinary biomarkers of 11β-HSD1 activity, that is, (αTHF + THF)/THE and THF/THE, were decreased by an order of magnitude, indicating 11β-HSD1 inhibition (Figure 2c,d and Table S1).

The parameters reported previously (Othonos et al., 2023; Table S1) were used to test possible correlations with AZD4017 treatment and to assess the efficacy of the proposed bile acid ratio as biomarker of 11β-HSD1 inhibition. Possible correlations between the GUDCA/G7oxoLCA and GCDCA/G7oxoLCA ratios with the clinical variables shown in Table S1 were investigated (see Table S4). As expected, no correlations and no influence of any parameter on these two bile acid ratios could be found for the placebo and AZD4017 baseline groups. Importantly, Spearman correlations between the GUDCA/G7oxoLCA ratio and the urinary THF/THE and (αTHF + THF)/THE ratios revealed strong positive associations, with $r = 0.81$ and $r = 0.85$, respectively, when comparing placebo and AZD4017 Post-Administration groups. Furthermore, GUDCA/G7oxoLCA correlated negatively with serum DHEAS ($r = -0.58$) and androstenedione ($r = -0.64$) and with urinary cortisone ($r = -0.65$) and cortisol ($r = -0.69$). Moreover, the concentration of the inhibitor AZD4017 was used to evaluate its influence on the observed bile acid changes, and as predicted, a negative correlation was obtained ($r = -0.77$). Similar results were obtained for the GCDCA/G7oxoLCA ratio (Table S4).

3.2 | Validation of the bile acid ratios as biomarkers of 11β-HSD1 inhibition in females with idiopathic intracranial hypertension (IIH) (cohort B)

To confirm the predictive value of the GUDCA/G7oxoLCA and GCDCA/G7oxoLCA ratios for the pharmacological inhibition of 11β-

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TABLE 2 Concentrations of individual bile acids in plasma of clinical cohort B.

Cohort B	Placebo Baseline (n = 12) nmol·L^{-1} (mean ± SD)	AZD4017 Baseline (n = 17) nmol·L^{-1} (mean ± SD)	Placebo Post-Administration (n = 12) nmol·L^{-1} (mean ± SD)	AZD4017 Post-Administration (n = 17) nmol·L^{-1} (mean ± SD)
Unconjugated				
CA	149 ± 275	93 ± 74	114 ± 152	329 ± 592
CDCA	260 ± 363	157 ± 134	241 ± 266	258 ± 523
DCA	422 ± 192	454 ± 406	406 ± 218	452 ± 410
UDCA	85 ± 129[a]	36 ± 56[a]	79 ± 91[a]	35 ± 45[a]
7oxoLCA	5.0 ± 4.3[a]	3.2 ± 0.1[a]	6.7 ± 5.4[a]	8.0 ± 12.0[a]
12oxoLCA	10.1 ± 16.1[a]	9.4 ± 12.3[a]	7.9 ± 6.8[a]	9.2 ± 13.6[a]
Conjugated				
GCA	394 ± 364	226 ± 188	235 ± 205	261 ± 147
GCDCA	1259 ± 1207	861 ± 874	792 ± 658	859 ± 509
GDCA	585 ± 592	376 ± 431	382 ± 303	466 ± 398
GUDCA	236 ± 319	113 ± 96	191 ± 129	97 ± 97
G7oxoLCA	0.2 ± 0[a]	0.2 ± 0[a]	0.2 ± 0[a]	4.4 ± 6.7[*,†,‡]
GLCA	37.9 ± 58.7	26.6 ± 33.9	17.3 ± 12.1	21.6 ± 26.2
TCA	47.5 ± 87.6[a]	11.5 ± 25.8[a]	20.5 ± 24.5[a]	17.4 ± 42.7[a]
TCDCA	198 ± 213	118 ± 129	85 ± 47	138 ± 102
TDCA	103 ± 146	40.5 ± 30.6	41.9 ± 38.3	63.9 ± 76.5
Total unconjugated	931 ± 727	753 ± 558	855 ± 547	1090 ± 1199
Total conjugated	2861 ± 2593	1773 ± 1500	1764 ± 1204	1929 ± 944
Total bile acids	3791 ± 2595	2526 ± 1760	2619 ± 1194	3019 ± 1810
Ratios				
GCDCA/G7oxoLCA	7040 ± 6746	4811 ± 4889	4429 ± 3676	1653 ± 2410[†,‡]
GUDCA/G7oxoLCA	1318 ± 1784	629 ± 535	1067 ± 723	208 ± 501[*,†,‡]

Note: Unequal samples sizes are the result of the exclusion of two subject pre- and post-administration of placebo due to limited sample amount. Bile acids were quantified by LC-MS/MS (Gómez et al., 2020). The GUDCA/G7oxoLCA and GCDCA/G7oxoLCA ratios were tested as potential biomarkers of 11β-HSD1 inhibition. Values are expressed as mean ± SD (nmol·L^{-1}).

[a]Not all of the values were within the limit of quantification (LOQ); the value LOQ/2 was assigned for samples that were below LOQ. No outliers were excluded.

*$P \leq 0.01$ for Placebo Baseline versus AZD4017 Post-Administration groups.

†$P \leq 0.01$ for AZD4017 Baseline versus AZD4017 Post-Administration groups.

‡$P \leq 0.01$ for Placebo Post-Administration versus AZD4017 Post-Administration groups.

HSD1, a second clinical cohort B, consisting of a group of 29 women diagnosed with active idiopathic intracranial hypertension (IIH) (intracranial pressure [ICP] > 25 cmH_2O) and active papilloedema, was investigated. Plasma samples from cohort B, collected before and after administration of placebo or AZD4017, were analysed by LC–MS/MS to determine bile acid profiles (Gómez et al., 2020). The mean concentrations of individual bile acids and the sums of unconjugated, conjugated and all measured bile acids are shown in Table 2. With the exception of G7oxoLCA, which was significantly increased in the AZD4017 Post-Administration group, there were no significant changes in any of the bile acids analysed when comparing the four groups. G7oxoLCA levels were below LOQ in most of the samples analysed, except in the AZD4017 Post-Administration group, and to estimate the GCDCA/G7oxoLCA and GUDCA/G7oxoLCA ratios shown in Table 2, the value LOQ/2 was used for the samples that could not be quantified. The GUDCA/G7oxoLCA ratio was significantly decreased in the AZD4017 Post-Administration group compared to the other groups, while the GCDCA/G7oxoLCA ratio did not reach significance (Table 2).

To reach sufficient sensitivity for quantification of G7oxoLCA also in the control groups, the shorter and focused LC–MS/MS method was applied. The mean concentrations of GUDCA, GCDCA and G7oxoLCA and the GUDCA/G7oxoLCA and GCDCA/G7oxoLCA ratios are shown in Table S5. G7oxoLCA levels were significantly increased in the AZD4017 Post-Administration group compared to the three other groups. Additionally, the GCDCA/G7oxoLCA and GUDCA/G7oxoLCA ratios were significantly decreased in the AZD4017 Post-Administration group compared to the other groups (Figure 3a,b and Table S5).

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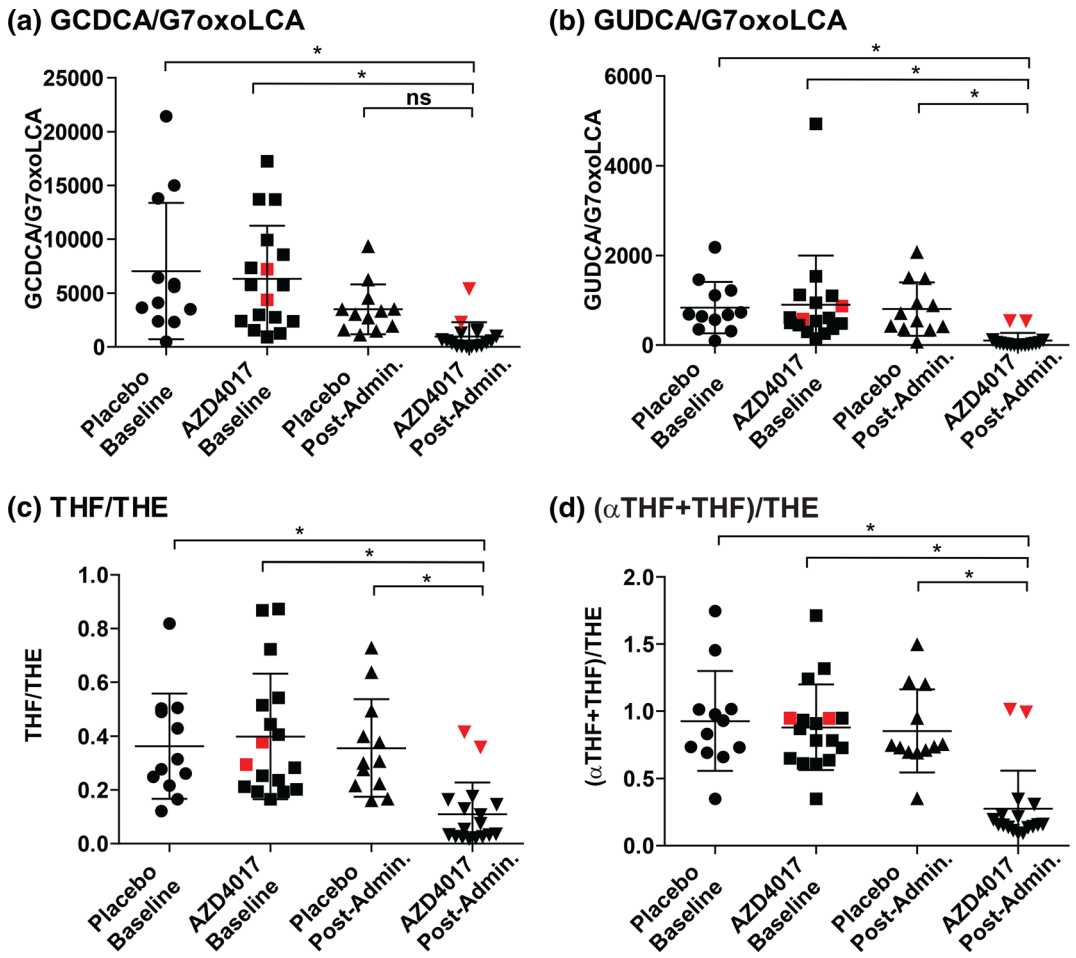

FIGURE 3 Plasma bile acid ratios and urine glucocorticoid metabolite ratios as biomarkers of 11β-HSD1 inhibition. The ratios GCDCA/G7oxoLCA (a) and GUDCA/G7oxoLCA (b) in human plasma from cohort B were determined by quantification of the respective bile acids by LC–MS/MS. The ratios THF/THE (c) and (αTHF + THF)/THE (d) in 24-h urine samples from cohort B were determined by GC–MS as reported earlier (Markey et al., 2020). Values are expressed as mean ± SD. Two non-responders are highlighted in red. *$P < 0.01$. No outliers were excluded.

Different clinical parameters measured in serum of cohort B were reported earlier (Hardy et al., 2021; Markey et al., 2020) and are shown in Table S2. Blood lipids and parameters of liver function were not different among the four groups. However, while concentrations of serum androgens and glucocorticoids were comparable between the four groups, there was a trend to decrease of the cortisol/DHEA and cortisol/cortisone ratios in the ADZ4017 treatment group, along with significantly decreased urinary THF/THE and (αTHF + THF)/THE ratios as markers of 11β-HSD1 activity (Figure 3c,d and Table S2) (Markey et al., 2017, 2020). The cortisone metabolite THE was significantly increased, and THF and αTHF tended to be decreased in 24-h urine samples of the AZD4017 Post-Administration group compared to the baseline and placebo treated groups.

Possible correlations of the GCDCA/G7oxoLCA and GUDCA/G7oxoLCA ratios with the available clinical parameters shown in Table S2 (Markey et al., 2020) were examined (see Table S6). As expected, no correlations could be found between the two bile acid ratios and any of the clinical parameters for the placebo and AZD4017 baseline groups. Then, Spearman correlations between the two bile acid ratios and the remaining variables were investigated in both Post-Administration groups (placebo vs. AZD4017). The GUDCA/G7oxoLCA ratio correlated positively with the urinary ratios THF/THE ($r = 0.77$) and (αTHF + THF)/THE ($r = 0.73$) and the serum ALP ($r = 0.50$) and cortisol/cortisone ratio ($r = 0.58$). Moreover, the concentration of the inhibitor AZD4017 correlated negatively with the GUDCA/G7oxoLCA ratio ($r = -0.76$). Similar results were obtained for the GCDCA/G7oxoLCA ratio (Table S6).

3.3 | Quantification of AZD4017 concentrations in cohorts A and B

To detect potential outliers in the treatment group, an LC–MS/MS method was established for the quantification of the 11β-HSD1 inhibitor AZD4017 in the placebo and AZD4017 Post-Administration groups of cohorts A and B. As expected, the inhibitor was absent in all samples from the placebo Post-Administration groups. In the AZD4017 Post-Administration group of cohort A, the 11β-HSD1

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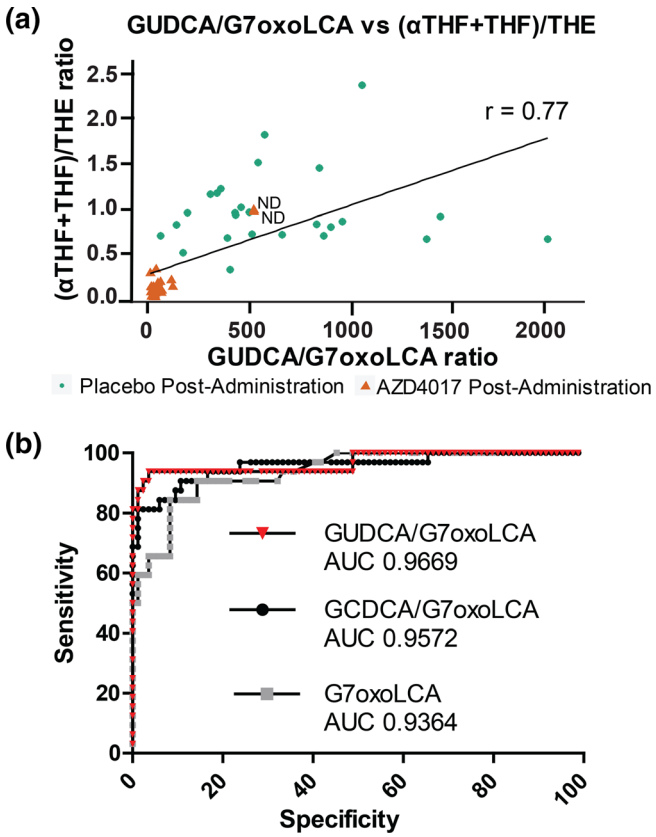

FIGURE 4 Contrasting data from AZD4017 treated individuals with those of untreated controls. (a) Spearman correlation of the urinary (αTHF + THF)/THE ratio and the blood GUDCA/G7oxoLCA ratio ($r = 0.77$), combining data from placebo and AZD4017 Post-Administration groups from both cohorts A and B. Two individuals with non-detectable AZD4017 levels are marked by ND. (b) Receiver operating characteristic (ROC) analyses for GUDCA/G7oxoLCA, GCDCA/G7oxoLCA and G7oxoLCA comparing data from placebo and pre-AZD4017 with the post-AZD4017 administration groups from both cohorts A and B. Sensitivity in % is plotted against specificity in %. No outliers were excluded.

inhibitor was detected in all serum samples (n = 15), with concentrations of 894 ± 497 nmol·L^{-1} (mean ± SD). In cohort B, the inhibitor could be detected in all but two plasma samples from individuals of the AZD4017 Post-Administration group (n = 17), with concentrations of 1384 ± 1152 nmol·L^{-1} (mean ± SD). The two participants of the AZD4017 Post-Administration group with non-detectable blood inhibitor levels also showed the highest bile acid and tetrahydroglucocorticoid metabolite ratios of their group, as highlighted in red in Figures 3 and S1 and marked as ND in Figures 4a and S2.

3.4 | Robustness of the proposed bile acid ratio biomarkers of 11β-HSD1 activity

To analyse the robustness of the proposed bile acid ratio biomarkers of 11β-HSD1 activity, the results obtained from cohort A and cohort B were combined. Despite the considerable differences in the design of the two cohorts in terms of sex, health state, BMI, time of treatment, co-treatment and matrix to be analysed, the blood bile acid ratios GCDCA/G7oxoLCA and GUDCA/G7oxoLCA as well as the THF/THE and (αTHF + THF)/THE ratios determined in 24-h urine samples were significantly decreased in the AZD4017 Post-Administration groups of both cohorts A and B and of the combined data thereof, even without excluding the two outliers of cohort B marked in red (Figures 3 and S1). Additionally, a principal component analysis (PCA) was performed by combining the data of all samples from cohorts A and B and from baseline and Post-Administration groups. All available clinical parameters, the urinary tetrahydroglucocorticoid ratios and the bile acid ratios were used for the analysis (Figure S2). The principal component analysis (PCA) analysis clearly separated the ADZ4017 Post-Administration group from the other three control groups. The two outliers with non-detectable drug levels are indicated as ND and they clustered with the control groups.

The GUDCA/G7oxoLCA ratio is easily accessible from blood samples and, in contrast to the 5-reduced glucocorticoid metabolites ratio, not affected by the glucocorticoid metabolizing enzymes SRD5A, AKR1D1 and 11β-HSD2. Thus, we aimed to compare the novel human blood biomarker GUDCA/G7oxoLCA of 11β-HSD1 activity with the currently applied (THF + αTHF)/THE ratio in 24 h urine samples. Despite the high heterogeneity of the two cohorts investigated, the data from the placebo and AZD4017 Post-Administration groups from both cohorts A and B revealed a strong correlation ($r = 0.77$) (Figure 4a). The values from the AZD4017 Post-Administration group clustered together, with the exception of the two individuals with non-detectable plasma AZD4017 levels (marked as ND).

As product/substrate ratios are often more reliable biomarkers than individual metabolite concentrations, the predictive potential of the GUDCA/G7oxoLCA ratio was compared with that of G7oxoLCA by conducting receiver operating characteristic (ROC) analyses (Figure 4b). The GUDCA/G7oxoLCA ratio performed better than the GCDCA/G7oxoLCA ratio or the absolute G7oxoLCA concentration. Using Youden's J-statistic yielded a threshold of ≤134 for GUDCA/G7oxoLCA, ≤727 for GCDCA/G7oxoLCA and >0.445 nM for G7oxoLCA, respectively.

4 | DISCUSSION

Inhibition of 11β-HSD1 is considered for treatment of various diseases including metabolic disorders, atherosclerosis, idiopathic intracranial hypertension (IIH), skin diseases, osteoporosis, cognitive impairments in ageing and optimization of glucocorticoid therapy to ameliorate adverse effects in metabolic target organs (Anderson & Walker, 2013; Bianzano et al., 2021; Chuanxin et al., 2020; Courtney et al., 2008; Feig et al., 2011; Freude et al., 2016; Gregory et al., 2020; Heise et al., 2014; Markey et al., 2020; Othonos et al., 2023; Schwab et al., 2017; Shah et al., 2011; Stefan et al., 2014; Webster et al., 2017). Non-invasive biomarkers to assess the activity of a given target can greatly facilitate the evaluation of drug efficacy. With respect to 11β-HSD1, ratios of urinary glucocorticoid

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metabolites, that is, THF/THE and (5αTHF + THF)/THE, are usually used in clinical studies to assess the efficacy of inhibitors of this enzyme (Bianzano et al., 2021; Courtney et al., 2008; Freude et al., 2016; Jamieson et al., 1999; Markey et al., 2020; Sagmeister et al., 2019; Tomlinson & Stewart, 2001; Webster et al., 2017). However, this requires an additional intervention, namely, the collection of 24-h urine samples, and leads to a higher burden for the patient and additional costs. A blood-based biomarker is currently not available; however, a recent preclinical study using four different mouse models proposed the use of the bile acid ratio TUDCA/T7oxoLCA as a blood biomarker for genetically or pharmacologically decreased 11β-HSD1 oxoreductase activity (Weingartner et al., 2021). As a next step, the present study aimed at translating this bile acid ratio biomarker from mouse to human and it also assessed whether 11β-HSD1 inhibition would result in pronounced disturbances of the plasma bile acid profile.

Two clinical studies of pharmacological 11β-HSD1 inhibition were analysed. Both used the same inhibitor but differed in several important parameters, that is, health state (idiopathic intracranial hypertension vs. healthy), body weight (obese vs. normal weight), sex, co-medication (w/wo prednisolone) and the matrix of the sample (serum vs. plasma). Despite the differences in these parameters, the bile acid profiles in both cohorts revealed no gross alterations following 11β-HSD1 inhibition, with the exception of significantly increased levels of the secondary bile acid G7oxoLCA. Earlier studies using transgenic mice lacking 11β-HSD1 activity observed elevated plasma bile acid concentrations (Penno et al., 2014; Weingartner et al., 2021), raising potential concerns for a higher risk of cholestasis upon inhibition of 11β-HSD1. Additionally, changes in the composition of the gut microbiota have been observed in 11β-HSD1 KO mice that could account for alterations in bile acid homeostasis (Johnson et al., 2017). The present study, including two different clinical cohorts and focusing on pharmacological inhibition, does not support such concerns, and the increase in G7oxoLCA was the major change in the bile acid profile following 11β-HSD1 inhibition. In humans, glycine conjugation is preferred over taurine conjugation, and T7oxoLCA was below the quantification limit. While the bile acid profiling method allowed for quantification of G7oxoLCA in samples from AZD4017 treated individuals, it was not sensitive enough to analyse this bile acid in the control groups. Thus, a shorter and focused LC–MS method specifically covering G7oxoLCA, GUDCA and GCDCA was developed and applied, enabling quantification of G7oxoLCA also in most of the samples from the control groups. For the few samples that still were undetectable or showed values below LOQ, the value LOQ/2 was included to calculate the ratios.

Although the inhibition of 11β-HSD1 resulted in increased G7oxoLCA blood levels, there are typically large interindividual variations when looking at a specific bile acid metabolite, likely due to differences in food intake and composition. Other factors such as age, sex, health state or co-medication can also influence the concentration of a specific bile acid. For these reasons product/substrate ratios are considered more reliable and robust biomarkers reporting altered enzyme activity. This was demonstrated by a recent preclinical study in 11β-HSD1 KO mice (Weingartner et al., 2021), where TUDCA/T7oxoLCA was shown to robustly detect genetically and pharmacologically diminished 11β-HSD1 activity, whereas the concentration of T7oxoLCA alone was elevated but showed high interindividual variation and was therefore not an ideal biomarker for the impaired enzyme activity. Thus, the bile acid ratios GUDCA/G7oxoLCA and GCDCA/G7oxoLCA were analysed as biomarkers of 11β-HSD1 oxoreductase activity in this study. While both ratios robustly detected the 11β-HSD1 inhibition, the GUDCA/G7oxoLCA ratio seemed to perform better. This may be explained by the fact that 7oxoLCA and its conjugated forms are preferentially converted to UDCA rather than CDCA and the respective conjugated metabolites (Odermatt et al., 2011; Penno et al., 2013). Furthermore, both 7oxoLCA and UDCA are secondary bile acids, whereas CDCA is a primary bile acid that is regulated by distinct physiological pathways.

A comparison of the use of the blood GUDCA/G7oxoLCA ratio with the 24-h urine THF/THE and (5αTHF + THF)/THE ratios showed that they all robustly detected the pharmacologically inhibited 11β-HSD1 activity. For both cohorts A and B, a positive correlation ($r > 0.7$) was found between the blood bile acid ratios and the urinary tetrahydro-glucocorticoid ratios tested. Importantly, quantification of blood AZD4017 levels revealed two individuals in cohort B with undetectable drug levels, suggesting either insufficient patient compliance or very rapid drug metabolism, and both showed blood bile acid ratios and 24-h urine tetrahydro-glucocorticoid ratios within the normal range. Interestingly, in cohort A, but not in cohort B, the GUDCA/G7oxoLCA ratio correlated negatively with serum DHEAS, serum androstenedione and urinary cortisone and cortisol concentrations ($r < -0.6$). In cohort A, AZD4017 was administered in combination with prednisolone, whereas the control group received only prednisolone. Prednisolone can be converted to the inactive prednisone by 11β-HSD2, mainly in the kidney, and regenerated by 11β-HSD1 in the liver and adipose. Prednisolone suppresses adrenal steroidogenesis via negative feedback regulation. Inhibition of 11β-HSD1 partially reversed the glucocorticoid-mediated suppression of adrenal steroidogenesis, explaining the negative correlation between these adrenal steroids and the blood bile acid and urinary tetrahydro-glucocorticoid ratios. To further assess the robustness of the GUDCA/G7oxoLCA ratio as a biomarker of pharmacological 11β-HSD1 inhibition, the data from both clinical cohorts were combined. Regardless of the differences between the two cohorts in terms of sex, health condition, BMI, co-medication or sample matrix, the values of the blood bile acid and urinary tetrahydro-glucocorticoid ratios obtained from both cohorts were in the same range and successfully detected the decreased 11β-HSD1 activity.

In conclusion, the present study proposes the GUDCA/G7oxoLCA ratio as a biomarker to detect pharmacological inhibition of 11β-HSD1. The main advantages offered by this blood bile acid ratio biomarker include its non-invasiveness, the need for small sample volume (25 μl of either plasma or serum that can be stored at −80°C until use), specificity and sensitivity due to mass spectrometry-based quantification, and that it is not influenced by 11β-HSD2, which does not accept CDCA, UDCA and their

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conjugates as substrates. There are several potential limitations that need to be addressed in future studies: (1) The GUDCA/G7oxoLCA ratio may mainly represent hepatic 11β-HSD1 oxoreductase activity. Whether decreased 11β-HSD1 oxoreductase activity specifically in skeletal muscle or adipose can also be detected remains to be determined, (2) it needs to be assessed whether co-medication, different diet or disease states can differentially affect GUDCA and G7oxoLCA, thereby lowering the informative value of the ratio, and (3) the GUDCA/G7oxoLCA ratio should be tested in disease situations where 11β-HSD1 and/or hexose-6-phosphate dehydrogenase (H6PD) are down-regulated to see whether it can detect the decreased oxoreductase activity.

AUTHOR CONTRIBUTIONS

Conceptualization: C. Gómez and A. Odermatt. *Methodology*: C. Gómez, Z. Alimajstorovic and N. Othonos. *Data acquisition and analysis*: C. Gómez, Z. Alimajstorovic and N. Othonos. *Validation*: C. Gómez. *Writing – original draft preparation*: C. Gómez. *Writing – review and editing*: C. Gómez, Z. Alimajstorovic, N. Othonos, D.V. Winter, S. White, G.G. Lavery, J.W. Tomlinson, A.J. Sinclair and A. Odermatt. *Supervision*: A. Odermatt. *Project administration*: A. Odermatt. *Funding acquisition*, A. Odermatt.

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CONFLICT OF INTEREST STATEMENT

A.J.S. reports personal fees from Invex Therapeutics in her role as Director with stock holdings as well as personal fees from Allergan, Novartis, Cheisi and Amgen (unrelated to the submitted work).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All related study data will be provided according to the related data management plan as open access at https://zenodo.org/.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design and Analysis, and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

ORCID

Cristina Gómez https://orcid.org/0000-0001-8518-0095
Alex Odermatt https://orcid.org/0000-0002-6820-2712

REFERENCES

Alexander, S. P. H., Cidlowski, J. A., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Coons, L., Fuller, P. J., Korach, K. S., & Young, M. J. (2021). The concise guide to pharmacology 2021/22: Nuclear hormone receptors. *British Journal of Pharmacology*, *178*, S246–S263. https://doi.org/10.1111/bph.15540

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Southan, C., Davies, J. A., Annett, S., Boison, D., Burns, K. E., Dessauer, C., Gertsch, J., Helsby, N. A., Izzo, A. A., … Wong, S. S. (2021). The concise guide to pharmacology 2021/22: Enzymes. *British Journal of Pharmacology*, *178*(S1), S313–S411. https://doi.org/10.1111/bph.15542

Anderson, A., & Walker, B. R. (2013). 11β-HSD1 inhibitors for the treatment of type 2 diabetes and cardiovascular disease. *Drugs*, *73*(13), 1385–1393. https://doi.org/10.1007/s40265-013-0112-5

Beck, K. R., Inderbinen, S. G., Kanagaratnam, S., Kratschmar, D. V., Jetten, A. M., Yamaguchi, H., & Odermatt, A. (2019). 11β-Hydroxysteroid dehydrogenases control access of 7β,27-dihydroxycholesterol to retinoid-related orphan receptor γ. *Journal of Lipid Research*, *60*(9), 1535–1546. https://doi.org/10.1194/jlr.M092908

Beck, K. R., Kanagaratnam, S., Kratschmar, D. V., Birk, J., Yamaguchi, H., Sailer, A. W., Seuwen, K., & Odermatt, A. (2019). Enzymatic interconversion of the oxysterols 7β,25-dihydroxycholesterol and 7-keto,25-hydroxycholesterol by 11β-hydroxysteroid dehydrogenase type 1 and 2. *The Journal of Steroid Biochemistry and Molecular Biology*, *190*, 19–28. https://doi.org/10.1016/j.jsbmb.2019.03.011

Bhat, B. G., Hosea, N., Fanjul, A., Herrera, J., Chapman, J., Thalacker, F., Stewart, P. M., & Rejto, P. A. (2008). Demonstration of proof of mechanism and pharmacokinetics and pharmacodynamic relationship with 4′-cyano-biphenyl-4-sulfonic acid (6-amino-pyridin-2-yl)-amide (PF-915275), an inhibitor of 11β- hydroxysteroid dehydrogenase type 1, in cynomolgus monkeys. *Journal of Pharmacology and Experimental Therapeutics*, *324*(1), 299–305. https://doi.org/10.1124/jpet.107.128280

Bianzano, S., Heise, T., Jungnik, A., Schepers, C., Schölch, C., & Gräfe-Mody, U. (2021). Safety, tolerability, pharmacokinetics and pharmacodynamics of single oral doses of BI 187004, an inhibitor of 11beta-hydroxysteroid dehydrogenase-1, in healthy male volunteers with overweight or obesity. *Clinical Diabetes and Endocrinology*, *7*(1), 16. https://doi.org/10.1186/s40842-021-00130-x

Cain, D. W., & Cidlowski, J. A. (2017). Immune regulation by glucocorticoids. *Nature Reviews Immunology*, *17*(4), 233–247. https://doi.org/10.1038/nri.2017.1

Chuanxin, Z., Shengzheng, W., Lei, D., Duoli, X., Jin, L., Fuzeng, R., Aiping, L., & Ge, Z. (2020). Progress in 11β-HSD1 inhibitors for the treatment of metabolic diseases: A comprehensive guide to their chemical structure diversity in drug development. *European Journal of Medicinal Chemistry*, *191*, 112134. https://doi.org/10.1016/j.ejmech.2020.112134

Courtney, R., Stewart, P. M., Toh, M., Ndongo, M. N., Calle, R. A., & Hirshberg, B. (2008). Modulation of 11β-hydroxysteroid dehydrogenase (11βHSD) activity biomarkers and pharmacokinetics of

14765381, 2024, 5, Downloaded from https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/bph.16251 by Nottingham Trent University, Wiley Online Library on [07/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

PF-00915275, a selective 11αHSD1 inhibitor. *Journal of Clinical Endocrinology and Metabolism*, *93*(2), 550–556. https://doi.org/10.1210/jc.2007-1912

Curtis, M. J., Alexander, S. P. H., Cirino, G., George, C. H., Kendall, D. A., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Patel, H. H., Sobey, C. G., Stanford, S. C., Stanley, P., Stefanska, B., Stephens, G. J., Teixeira, M. M., Vergnolle, N., & Ahluwalia, A. (2022). Planning experiments: Updated guidance on experimental design and analysis and their reporting III. *British Journal of Pharmacology*, *179*, 3907–3913. https://doi.org/10.1111/bph.15868

Feig, P. U., Shah, S., Hermanowski-Vosatka, A., Plotkin, D., Springer, M. S., Donahue, S., Thach, C., Klein, E. J., Lai, E., & Kaufman, K. D. (2011). Effects of an 11β-hydroxysteroid dehydrogenase type 1 inhibitor, MK-0916, in patients with type 2 diabetes mellitus and metabolic syndrome. *Diabetes, Obesity and Metabolism*, *13*(6), 498–504. https://doi.org/10.1111/j.1463-1326.2011.01375.x

Freude, S., Heise, T., Woerle, H.-J., Jungnik, A., Rauch, T., Hamilton, B., Schölch, C., Huang, F., & Graefe-Mody, U. (2016). Safety, pharmacokinetics and pharmacodynamics of BI 135585, a selective 11β-hydroxysteroid dehydrogenase-1 (HSD1) inhibitor in humans: Liver and adipose tissue 11β-HSD1 inhibition after acute and multiple administrations over 2 weeks. *Diabetes, Obesity and Metabolism*, *18*(5), 483–490. https://doi.org/10.1111/dom.12635

Gathercole, L. L., Lavery, G. G., Morgan, S. A., Cooper, M. S., Sinclair, A. J., Tomlinson, J. W., & Stewart, P. M. (2013). 11β-Hydroxysteroid dehydrogenase 1: Translational and therapeutic aspects. *Endocrine Reviews*, *34*(4), 525–555. https://doi.org/10.1210/er.2012-1050

Gómez, C., Stücheli, S., Kratschmar, D. V., Bouitbir, J., & Odermatt, A. (2020). Development and validation of a highly sensitive LC-MS/MS method for the analysis of bile acids in serum, plasma, and liver tissue samples. *Metabolites*, *10*(7), 282. https://doi.org/10.3390/metabo10070282

Gregory, S., Hill, D., Grey, B., Ketelbey, W., Miller, T., Muniz-Terrera, G., & Ritchie, C. W. (2020). 11β-Hydroxysteroid dehydrogenase type 1 inhibitor use in human disease—A systematic review and narrative synthesis. *Metabolism, Clinical and Experimental*, *108*, 154246. https://doi.org/10.1016/j.metabol.2020.154246

Hardy, R. S., Botfield, H., Markey, K., Mitchell, J. L., Alimajstorovic, Z., Westgate, C. S. J. J., Sagmeister, M., Fairclough, R. J., Ottridge, R. S., Yiangou, A., Storbeck, K.-H. H. H., Taylor, A. E., Gilligan, L. C., Arlt, W., Stewart, P. M., Tomlinson, J. W., Mollan, S. P., Lavery, G. G., & Sinclair, A. J. (2021). 11βHSD1 inhibition with AZD4017 improves lipid profiles and lean muscle mass in idiopathic intracranial hypertension. *Journal of Clinical Endocrinology and Metabolism*, *106*(1), 174–187. https://doi.org/10.1210/clinem/dgaa766

Heise, T., Morrow, L., Hompesch, M., Häring, H.-U., Kapitza, C., Abt, M., Ramsauer, M., Magnone, M.-C., & Fuerst-Recktenwald, S. (2014). Safety, efficacy and weight effect of two 11β-HSD1 inhibitors in metformin-treated patients with type 2 diabetes. *Diabetes, Obesity and Metabolism*, *16*(11), 1070–1077. https://doi.org/10.1111/dom.12317

Hult, M., Elleby, B., Shafqat, N., Svensson, S., Rane, A., Jörnvall, H., Abrahmsen, L., & Oppermann, U. (2004). Human and rodent type 1 11b-hydroxysteroid dehydrogenases are 7b-hydroxycholesterol dehydrogenases involved in oxysterol metabolism. *Cellular and Molecular Life Sciences: CMLS*, *61*(7–8), 992–999. https://doi.org/10.1007/s00018-003-3476-y

Jamieson, A., Wallace, A. M., Andrew, R., Nunez, B. S., Walker, B. R., Fraser, R., White, P. C., & Connell, J. M. C. (1999). Apparent cortisone reductase deficiency: A functional defect in 11β-hydroxysteroid dehydrogenase type 1. *Journal of Clinical Endocrinology and Metabolism*, *84*(10), 3570–3574. https://doi.org/10.1210/jcem.84.10.6031

Johnson, J. S., Opiyo, M. N., Thomson, M., Gharbi, K., Seckl, J. R., Heger, A., & Chapman, K. E. (2017). 11β-hydroxysteroid dehydrogenase-1 deficiency alters the gut microbiome response to Western diet. *Journal of Endocrinology*, *232*(2), 273–283. https://doi.org/10.1530/JOE-16-0578

Markey, K., Mitchell, J., Botfield, H., Ottridge, R. S., Matthews, T., Krishnan, A., Woolley, R., Westgate, C., Yiangou, A., Alimajstorovic, Z., Shah, P., Rick, C., Ives, N., Taylor, A. E., Gilligan, L. C., Jenkinson, C., Arlt, W., Scotton, W., Fairclough, R. J., … Sinclair, A. J. (2020). 11β-Hydroxysteroid dehydrogenase type 1 inhibition in idiopathic intracranial hypertension: A double-blind randomized controlled trial. *Brain Communications*, *2*(1), 1, fcz050–12. https://doi.org/10.1093/braincomms/fcz050

Markey, K. A., Ottridge, R., Mitchell, J. L., Rick, C., Woolley, R., Ives, N., Nightingale, P., & Sinclair, A. J. (2017). Assessing the efficacy and safety of an 11β-hydroxysteroid dehydrogenase type 1 inhibitor (AZD4017) in the idiopathic intracranial hypertension drug trial, IIH:DT: Clinical methods and design for a phase II randomized controlled trial. *JMIR Research Protocols*, *6*(9), e181. https://doi.org/10.2196/resprot.7806

Mitić, T., Shave, S., Semjonous, N., McNae, I., Cobice, D. F., Lavery, G. G., Webster, S. P., Hadoke, P. W. F., Walker, B. R., & Andrew, R. (2013). 11β-Hydroxysteroid dehydrogenase type 1 contributes to the balance between 7-keto- and 7-hydroxy-oxysterols in vivo. *Biochemical Pharmacology*, *86*(1), 146–153. https://doi.org/10.1016/j.bcp.2013.02.002

Odermatt, A., Da Cunha, T., Penno, C. A., Chandsawangbhuwana, C., Reichert, C., Wolf, A., Dong, M., & Baker, M. E. (2011). Hepatic reduction of the secondary bile acid 7-oxolithocholic acid is mediated by 11β-hydroxysteroid dehydrogenase 1. *Biochemical Journal*, *436*(3), 621–629. https://doi.org/10.1042/BJ20110022

Odermatt, A., Dick, B., Arnold, P., Zaehner, T., Plueschke, V., Deregibus, M. N., Repetto, H., Frey, B. M., Frey, F. J., & Ferrari, P. (2001). A mutation in the cofactor-binding domain of 11β-hydroxysteroid dehydrogenase type 2 associated with mineralocorticoid hypertension. *Journal of Clinical Endocrinology and Metabolism*, *86*(3), 1247–1252. https://doi.org/10.1210/jcem.86.3.7334

Odermatt, A., & Klusonova, P. (2015). 11β-Hydroxysteroid dehydrogenase 1: Regeneration of active glucocorticoids is only part of the story. *Journal of Steroid Biochemistry and Molecular Biology*, *151*, 85–92. https://doi.org/10.1016/j.jsbmb.2014.08.011

Odermatt, A., & Kratschmar, D. V. (2012). Tissue-specific modulation of mineralocorticoid receptor function by 11β-hydroxysteroid dehydrogenases: An overview. *Molecular and Cellular Endocrinology*, *350*(2), 168–186. https://doi.org/10.1016/j.mce.2011.07.020

Othonos, N., Pofi, R., Arvaniti, A., White, S., Bonaventura, I., Nikolaou, N., Moolla, A., Marjot, T., Stimson, R. H., van Beek, A. P., van Faassen, M., Isidori, A. M., Bateman, E., Sadler, R., Karpe, F., Stewart, P. M., Webster, C., Duffy, J., Eastell, R., … Tomlinson, J. W. (2023). 11β-HSD1 inhibition in men mitigates prednisolone-induced adverse effects in a proof-of-concept randomized double-blind placebo-controlled trial. *Nature Communications*, *14*(1), 1025. https://doi.org/10.1038/s41467-023-36541-w

Palermo, M., Shackleton, C. H. L., Mantero, F., & Stewart, P. M. (1996). Urinary free cortisone and the assessment of 11β-hydroxysteroid dehydrogenase activity in man. *Clinical Endocrinology*, *45*(5), 605–611. https://doi.org/10.1046/j.1365-2265.1996.00853.x

Penno, C. A., Morgan, S. A., Rose, A. J., Herzig, S., Lavery, G. G., & Odermatt, A. (2014). 11β-Hydroxysteroid dehydrogenase-1 is involved in bile acid homeostasis by modulating fatty acid transport protein-5 in the liver of mice. *Molecular Metabolism*, *3*(5), 554–564. https://doi.org/10.1016/j.molmet.2014.04.008

Penno, C. A., Morgan, S. A., Vuorinen, A., Schuster, D., Lavery, G. G., & Odermatt, A. (2013). Impaired oxidoreduction by 11β-hydroxysteroid dehydrogenase 1 results in the accumulation of 7-oxolithocholic acid. *Journal of Lipid Research*, *54*(10), 2874–2883. https://doi.org/10.1194/jlr.M042499

14765381, 2024, 5, Downloaded from https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/bph.16251 by Nottingham Trent University, Wiley Online Library on [07/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Reichardt, S. D., Amouret, A., Muzzi, C., Vettorazzi, S., Tuckermann, J. P., Lühder, F., & Reichardt, H. M. (2021). The role of glucocorticoids in inflammatory diseases. *Cell*, *10*(11), 2921. https://doi.org/10.3390/cells10112921

Russell, D. W., & Setchell, K. D. R. (1992). Bile acid biosynthesis. *Biochemistry*, *31*(20), 4737–4749. https://doi.org/10.1021/bi00135a001

Sagmeister, M. S., Taylor, A. E., Fenton, A., Wall, N. A., Chanouzas, D., Nightingale, P. G., Ferro, C. J., Arlt, W., Cockwell, P., Hardy, R. S., & Harper, L. (2019). Glucocorticoid activation by 11β-hydroxysteroid dehydrogenase enzymes in relation to inflammation and glycaemic control in chronic kidney disease: A cross-sectional study. *Clinical Endocrinology*, *90*, 241–249. https://doi.org/10.1111/cen.13889

Schwab, D., Sturm, C., Portron, A., Fuerst-Recktenwald, S., Hainzl, D., Jordan, P., Stewart, W. C., Tepedino, M. E., & DuBiner, H. (2017). Oral administration of the 11β-hydroxysteroid-dehydrogenase type 1 inhibitor RO5093151 to patients with glaucoma: An adaptive, randomised, placebo-controlled clinical study. *BMJ Open Ophthalmology*, *1*(1), e000063. https://doi.org/10.1136/bmjophth-2016-000063

Schweizer, R. A. S., Zürcher, M., Balazs, Z., Dick, B., & Odermatt, A. (2004). Rapid hepatic metabolism of 7-Ketocholesterol by 11β-hydroxysteroid dehydrogenase type 1. *Journal of Biological Chemistry*, *279*(18), 18415–18424. https://doi.org/10.1074/jbc.M313615200

Scott, J. S., Bowker, S. S., DeSchoolmeester, J., Gerhardt, S., Hargreaves, D., Kilgour, E., Lloyd, A., Mayers, R. M., McCoull, W., Newcombe, N. J., Ogg, D., Packer, M. J., Rees, A., Revill, J., Schofield, P., Selmi, N., Swales, J. G., & Whittamore, P. R. O. (2012). Discovery of a potent, selective, and orally bioavailable acidic 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitor: Discovery of 2-[(3 S)-1-[5-(cyclohexylcarbamoyl)-6-propylsulfanylpyridin-2-yl]-3-piperidyl]acetic acid (AZD4017). *Journal of Medicinal Chemistry*, *55*(12), 5951–5964. https://doi.org/10.1021/jm300592r

Scott, J. S., Goldberg, F. W., & Turnbull, A. V. (2014). Medicinal chemistry of inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). *Journal of Medicinal Chemistry*, *57*(11), 4466–4486. https://doi.org/10.1021/jm4014746

Shackleton, C. H. L. (1993). Mass spectrometry in the diagnosis of steroid-related disorders and in hypertension research. *The Journal of Steroid Biochemistry and Molecular Biology*, *45*(1–3), 127–140. https://doi.org/10.1016/0960-0760(93)90132-G

Shah, S., Hermanowski-Vosatka, A., Gibson, K., Ruck, R. A., Jia, G., Zhang, J., Hwang, P. M. T., Ryan, N. W., Langdon, R. B., & Feig, P. U. (2011). Efficacy and safety of the selective 11β-HSD-1 inhibitors MK-0736 and MK-0916 in overweight and obese patients with hypertension. *Journal of the American Society of Hypertension*, *5*(3), 166–176. https://doi.org/10.1016/j.jash.2011.01.009

Stefan, N., Ramsauer, M., Jordan, P., Nowotny, B., Kantartzis, K., Machann, J., Hwang, J.-H. H., Nowotny, P., Kahl, S., Harreiter, J., Hornemann, S., Sanyal, A. J., Stewart, P. M., Pfeiffer, A. F., Kautzky-Willer, A., Roden, M., Häring, H.-U. U., Fürst-Recktenwald, S., Pfeiff, A. F., & Kautzky-Willer, A. (2014). Inhibition of 11β-HSD1 with RO5093151 for non-alcoholic fatty liver disease: A multicentre, randomised, double-blind, placebo-controlled trial. *The Lancet Diabetes & Endocrinology*, *2*(5), 406–416. https://doi.org/10.1016/S2213-8587(13)70170-0

Tomlinson, J. W., & Stewart, P. M. (2001). Cortisol metabolism and the role of 11β-hydroxysteroid dehydrogenase. *Best Practice & Research. Clinical Endocrinology & Metabolism*, *15*(1), 61–78. https://doi.org/10.1053/beem.2000.0119

Webster, S. P., McBride, A., Binnie, M., Sooy, K., Seckl, J. R., Andrew, R., Pallin, T. D., Hunt, H. J., Perrior, T. R., Ruffles, V. S., Ketelbey, J. W., Boyd, A., & Walker, B. R. (2017). Selection and early clinical evaluation of the brain-penetrant 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibitor UE2343 (Xanamem™). *British Journal of Pharmacology*, *174*(5), 396–408. https://doi.org/10.1111/bph.13699

Weingartner, M., Stücheli, S., Kratschmar, D. V., Birk, J., Klusonova, P., Chapman, K. E., Lavery, G. G., & Odermatt, A. (2021). The ratio of ursodeoxycholyltaurine to 7-oxolithocholyltaurine serves as a biomarker of decreased 11β-hydroxysteroid dehydrogenase 1 activity in mouse. *British Journal of Pharmacology*, *178*, 3309–3326. https://doi.org/10.1111/bph.15367

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