


Altered cortisol metabolism in individuals with HNF1A-MODY

Agata Juszczak^{1,2}  | Lorna C. Gilligan³ | Beverly A. Hughes³ | Zaki K. Hassan-Smith³ | Mark I. McCarthy^{1,2,4} | Wiebke Arlt³ | Jeremy W. Tomlinson^{1,2} | Katharine R. Owen^{1,2}

¹Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Oxford, UK

²NIHR Oxford Biomedical Research Centre, Churchill Hospital, Oxford, UK

³Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, UK

⁴Wellcome Trust Centre for Human Genetics, Oxford, UK

Correspondence

Katharine Owen, Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Old Rd, OX3 7LE Oxford, UK.

Email: Katharine.owen@dr1.ox.ac.uk

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Abstract

Objective and context: Maturity onset diabetes of the young due to variants in *HNF1A* (HNF1A-MODY) is the most common form of monogenic diabetes. Individuals with HNF1A-MODY usually have a lean phenotype which contrasts with type 2 diabetes (T2DM). Data from hepatocytes derived from *Hnf1a* knock-out mice demonstrated dysregulation of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1), which regulates glucocorticoid availability and action in target tissues, together with 11 β -HSD2 and steroid A-ring reductases, 5 α - and 5 β -reductase. We proposed that altered glucocorticoid metabolism might underpin some of the phenotypic differences between patients with HNF1A-MODY and those with T2DM.

Design: A retrospective matched cohort study.

Patients and measurements: 24-hours urine steroid metabolome profiling was carried out by gas chromatography-mass spectrometry in 35 subjects with HNF1A-MODY, 35 individuals with T2DM and 35 healthy controls matched for age, sex and BMI. The steroid metabolites were expressed as $\mu\text{g/L}$ in all groups and measured in mid-morning urine in diabetic subjects and 24-hour urine collection in healthy controls. Hence, only ratios were compared not the individual steroids. Established ratios of glucocorticoid metabolites were used to estimate 11 β -HSD1/2 and 5 α - and 5 β -reductase activities.

Results: While 11 β -HSD1 activity was similar in all groups, 11 β -HSD2 activity was significantly lower in subjects with HNF1A-MODY and T2DM than in healthy controls. The ratio of 5 β - to 5 α -metabolites of cortisol was higher in subjects with HNF1A-MODY than in T2DM and healthy controls, probably due to increased activity of the 5 β -reductase (AKR1D1) in HNF1A-MODY.

Conclusions: This is the first report of steroid metabolites in HNF1A-MODY. We have identified distinct differences in steroid metabolism pathways in subjects with HNF1A-MODY that have the potential to alter steroid hormone availability. Further studies are required to explore whether these changes link to phenotype.

KEYWORDS

HNF1A, MODY, urinary steroids

1 | INTRODUCTION

Maturity onset diabetes of the young (MODY) due to inactivating alleles in *HNF1A* is the most common form of monogenic diabetes. *HNF1A* (hepatocyte nuclear factor 1-alpha) acts as a transcription factor for more than 200 genes and is expressed in the liver, pancreas, gut and kidneys. Low expression is reported in the testis and female genital tract but none in the adrenals.¹ Individuals with *HNF1A*-MODY are usually slim and sensitive to small doses of sulphonylurea² but also more sensitive to insulin when compared with those with type 2 diabetes mellitus (T2DM).³ Whilst the genetic basis for the disease has been established, the pathogenic processes that contribute to its phenotype have not been fully elucidated.

Circulating glucocorticoid excess is an established cause of a severe adverse metabolic phenotype. This is best exemplified by patients with Cushing's syndrome, who develop florid central obesity, hypertension, insulin resistance and in some cases overt T2DM. However, simple obesity and T2DM are not states of circulating glucocorticoid excess.⁴ At a tissue-specific level, the availability of cortisol to bind and activate the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) is controlled by a series of enzymes that either inactivate or reactivate cortisol, known as 'pre-receptor' metabolism.

11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) interconverts inactive cortisone to active cortisol, predominantly activating cortisone to cortisol via its reductase activity (Figure 1),⁵ and is mainly expressed in the liver and adipose tissue. 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) unidirectionally inactivates cortisol to cortisone in mineralocorticoid target tissues, for example placenta, kidney and colon.^{1,6} Cortisol binds to the mineralocorticoid receptor with similar affinity to aldosterone, but 11 β -HSD2

protects the MR from cortisol occupancy by rapidly inactivating cortisol to cortisone, therefore enabling aldosterone to act as the major mineralocorticoid.⁷

Cortisol can also be metabolized to 5 α - or 5 β -reduced metabolites by 5 α - and 5 β -reductases (SRD5A1, SRD5A2 and AKR1D1).⁸ SRD5A1 is expressed abundantly in most tissues in humans. SRD5A2 is mainly expressed in the liver and genital tract, while AKR1D1 is expressed in the liver with low expression in the testes and Fallopian tubes.¹ The A-ring reductases and 11 β -HSD1 have been implicated in the pathogenesis of metabolic disease. In rodent studies, selective 11 β -HSD1 inhibitors improved glucose tolerance and insulin sensitivity, but clinical studies have shown less impressive results.^{9,10} Phase II studies of selective 11 β -HSD1 inhibitors in obese human subjects with T2DM described only a modest effect on hyperglycaemia and body weight (FPG reduced by 0.3-1.3 mmol/L, HbA1c decreased by 0.56%, body weight reduced by 1-1.8 kg).^{11,12}

Altered A-ring reductase activity has also been implicated in the development of an adverse metabolic phenotype. Elevated global 5 α -reductase activity with increased 5 α -metabolites of cortisol has been reported in obese human subjects with biopsy-proven fatty liver when compared to BMI-matched individuals.¹³ Furthermore, non-selective inhibition of both 5 α -reductase isoforms using dutasteride, an inhibitor of SRD5A1 and SRD5A2, worsened metabolic phenotype, putatively through reduced glucocorticoid clearance.^{14,15}

Genes encoding for several enzymes in steroid metabolism were reported to be downregulated in hepatocytes from *Hnf1a* knock out (-/-) mice.¹⁶ This included 11 β -HSD1 (*Hsd11b1*). The expression of this and 7 other enzymes involved in the steroid metabolism was analysed only in hepatocytes and islets by Servitja et al and expression in adipose tissue was not reported. The expression of *Hsd11b1* in hepatocytes from the *Hnf1a* -/- mice was downregulated 15-fold when compared to the wild type (WT). Based on the data from *Hnf1a* knock-out mice, we anticipated a decreased expression and activity of 11 β -HSD1 in subjects with *HNF1A*-MODY, leading to decreased cortisol exposure locally and therefore potentially resulting in a relatively beneficial metabolic phenotype in comparison with the individuals with T2DM.

The exploration of the urine steroid metabolome has provided novel mechanistic insights and novel biomarkers for some diseases,^{17,18} and the steroid ratios can be used as a reflection of distinct enzymatic activities. In this study, we examined the urinary steroid profile in individuals with *HNF1A*-MODY and T2DM to see if it would offer insight into the phenotype observed in MODY.

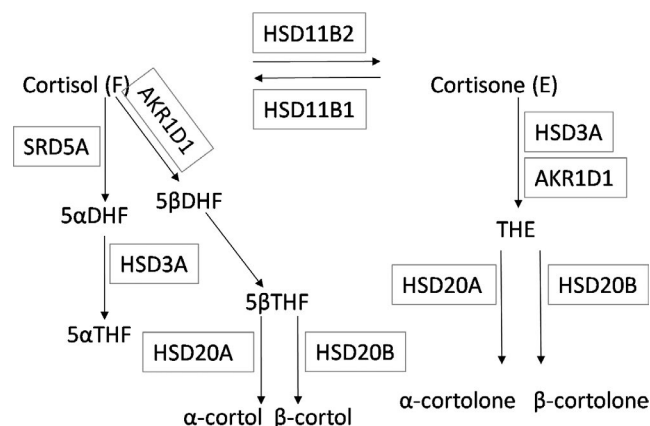


FIGURE 1 Metabolism of cortisol; grey shaded boxes include ratios used for estimating activity of HSD11B1, HSD11B2 and A-ring reductases (SRD5A and AKR1D1); HSD11B1 and HSD11B2, 11 β -hydroxysteroid dehydrogenase type 1 and 2; SRD5A and AKR1D1, 5 α - and 5 β -steroid reductase; F, cortisol; E, cortisone; DHF, dihydrocortisol; THF, tetrahydrocortisol; THE, tetrahydrocortisone; HSD3A, 3 α -hydroxysteroid dehydrogenase; HSD20A, 20 α -hydroxysteroid dehydrogenase; HSD20B, 20 β -hydroxysteroid dehydrogenase

2 | STUDY PARTICIPANTS

35 individuals with *HNF1A*-MODY, 35 with T2DM and 35 healthy controls were recruited for the study. Clinical information on age of diabetes onset, treatment history, current medications and family history of diabetes were recorded and anthropometrical measurements collected. Basic metabolic parameters such as HbA1c, fasting

plasma glucose, C-peptide, renal function and lipid profile were measured. Subjects with both types of diabetes were consecutively selected from the YDX (Young Diabetes in Oxford) study which recruited individuals with diabetes onset below 45 years of age and above 18 years at the time of recruitment from Thames Valley primary and secondary care centres. Healthy control participants were selected from the Healthy Human Aging study from the local Birmingham population.¹⁹ These subjects had fasting plasma glucose measured to exclude undiagnosed diabetes and did not have history of IHD, CVD, respiratory disease or epilepsy. Both studies had local ethics committee approval (REC reference 04/Q1604/97 and REC reference 07/H1211/168, respectively).

Participants were divided further into sex-specific groups due to the established difference in the urinary steroid profile between genders,²⁰ with recruitment of similar numbers of men and women to facilitate sex-specific analysis. The groups were matched for BMI and age. First, mean age and BMI of HNF1A-MODY subjects were calculated in both gender groups, then subjects from YDX with T2DM and healthy controls were selected to match BMI and age of the first group. Spot morning urine samples were collected from all diabetic individuals, while healthy controls provided 24-hour urine collections. Female subjects were not on any contraceptive or hormonal replacement therapy, and individuals on any form of steroid treatment were excluded. One subject from the HNF1A-MODY group was retrospectively excluded due to a serum creatinine above 160 µmol/L.

3 | METHODS

The urinary steroid profile was measured in a mid-morning spot urine in subjects with diabetes, and the values were expressed in µg/L of urine. The values from 24-hours urine collection in the healthy controls were also converted to µg/L of urine.

Thirty-two different urinary steroids and their metabolites were analysed by gas chromatography-mass spectrometry (GC-MS) selected-ion monitoring analysis (Table 2) as described previously.²² In brief, free and conjugated steroids were extracted from 1 mL of urine by solid-phase extraction. Steroid conjugates were enzymatically hydrolysed, re-extracted and chemically derivatized to form methyloxime trimethyl silyl ethers. GC-MS was performed on an Agilent 5975 instrument operating in selected-ion-monitoring (SIM) mode to achieve sensitive and specific detection and quantification of steroid metabolites including androgens, glucocorticoids and mineralocorticoids.

Because of the difference in the way urine was collected between diabetic subjects and healthy controls, we elected to compare only ratios of urinary steroids and individual or total corticosteroids metabolites were not discussed. The activity of 11β-HSD1 was estimated by the ratio of urinary metabolites of cortisol (F) to the metabolite of cortisone (E): (5βTHF + 5αTHF)/THE, where THF is tetrahydrocortisol; THE, tetrahydrocortisone.²³ The ratios of F/E and F + E/(5βTHF + 5αTHF)+THE were used to estimate the function

of 11β-HSD2. The ratios of 5βTHF/ 5αTHF and etiocholanolone/ androsterone defined the total 5α-reductase activity (conveyed by SRD5A1 and SRD5A2) and total 5β-reductase activity (conveyed by AKR1D1). Figure 1 shows a simplified schematic overview of cortisol metabolism with the relevant steroids and enzymes included.

Urinary steroid ratio (5βTHF + 5αTHF)/THE measured in spot mid-morning urine samples were previously reported to correlate well with urinary steroids measured in urine from 24-hour collection.²¹ We analysed data from the same group of six healthy men (mean age 33 ± 7 years with BMI in the normal range) and confirmed that 5βTHF/5αTHF and etiocholanolone/androsterone ratios represented good agreement with a concordance correlation coefficient (CCC) of 0.94 for the first ratio and 0.98 for the second ratio. The ratio used for the assessment of HSD11B1 activity, (5βTHF + 5αTHF)/THE, also showed good agreement with CCC of 0.94. However, in this small group of healthy volunteers, the agreement was low for ratios used to describe activity of HSD11B2 (CCC 0.25 and -0.06 for F/E and F + E/(5βTHF + 5αTHF)+THE), respectively).

3.1 | Statistical analysis

IBM SPSS v23 was used for statistical analysis. Patient characteristics were expressed as mean (±SD) or median (IQR) depending on the distribution of continuous data and as percentages for categorical measures. Differences of frequencies for categorical variables were tested using the chi-squared test. Most of the urinary steroid ratios had a nonparametric distribution and thus were expressed as median (IQR), and *P* values were calculated using the Mann-Whitney U test when comparing HNF1A-MODY patients with two other groups separately. Significant differences were considered for *P* < .032, which was calculated using the Benjamini-Hochberg procedure for a total of 49 metabolites/ratios analysed and an accepted false discovery rate of 10%. A significant correlation was defined by a *P* value of <.05 using Pearson's correlation coefficient.

4 | RESULTS

4.1 | Patient characteristics

All subjects were age- and BMI-matched in each gender group (mean age in all 3 groups was 38.8 [±9.0] years in men, 45.2 [±14.2] years in women; BMI 26.5 [±3.5] and 26.5 [±2.7] kg/m², respectively) (Table 1). However, women with T2DM had a higher mean waist-to-hip ratio when compared to those with HNF1A-MODY (0.93 [±2.27] vs 0.82 [±0.06], *P* < .0001) despite matching for BMI in both groups. There was no difference in waist-to-hip ratio in men with both types of diabetes (Table 1). There was no significant difference in creatinine level between HNF1A-MODY and T2DM groups. All healthy subjects had a urinary creatinine measurement in the normal range.

The HbA1c and total cholesterol were similar in both groups with diabetes (Table 1). The median duration of diabetes was longer in

TABLE 1 Clinical characteristics of the three studied groups comprising patients with HNF1A-MODY, T2DM and healthy controls, respectively

	Study group			P value	
	HNF1A-MODY	T2D	Control	HNF1A vs T2D	HNF1A vs Control
Men					
Number of subjects	n = 16	n = 18	n = 17		
Mean (SD) age [years]	39.43 (11.16)	38.49 (5.99)	38.41 (9.97)	.83	.85
Mean (SD) BMI [kg/m ²]	26.92 (4.61)	26.69 (3.17)	25.83 (2.56)	.75	.49
Mean (SD) Waist/hip ratio	0.91 (0.06)	0.91 (0.08)	n/a	.88	n/a
Median (IQR) systolic BP [mm Hg]	139 (130.5-148.5)	136 (121-139)	125 (117-128)	.39	.02
Median (IQR) diastolic BP [mm Hg]	84 (72.5-89.5)	81 (76-85)	80 (72-85)	.88	.50
Mean (SD) creatinine [μmol/L]	84.78 (13.82)	81.89 (23.79)	n/a	.20	n/a
Mean (SD) K ⁺ [mmol/L]	4.02 (0.47)	4.28 (0.57)	n/a	.22	n/a
Median (IQR) onset of diabetes [years]	24.50 (20.50-33.50)	35.00 (28.00-38.00)	n/a	.07	n/a
Median (IQR) DM duration [years]	11.26 (7.65-18.60)	2.96 (1.26-13.00)	n/a	<.01	n/a
Median (IQR) HbA1c [%]	7.20 (6.35-7.80)	6.70 (6.50-7.30)	n/a	.81	n/a
Median (IQR) FPG [mmol/L]	6.70 (6.20-8.60)	7.20 (6.30-9.50)	5.10 (4.80-5.10)	.56	<.0001
Median (IQR) HOMA2-IR	1.37 (0.64-1.67)	1.63 (1.42-2.19)	1.17 (0.71-1.91)	.04	.81
Mean (SD) total cholesterol [mmol/L]	4.80 (0.97)	4.60 (0.99)	4.76 (1.13)	.47	.74
Mean (SD) HDL [mmol/L]	1.29 (0.45)	1.02 (0.20)	1.27 (0.21)	.10	.41
Insulin treated	5 (31.3%)	4 (22.2%)	n/a	.24	n/a
Statin treated	4 (25%)	7 (38.9%)	n/a	.31	n/a
HTN treated	2 (12.5%)	7 (38.9%)	n/a	.07	n/a
Women					
Number of subjects	n = 18	n = 18	n = 18		
Mean (SD) age [years]	44.96 (16.32)	48.42 (10.34)	42.17 (16.07)	.67	.61
Mean (SD) BMI [kg/m ²]	26.74 (2.58)	26.55 (2.27)	26.16 (3.38)	.55	.39
Mean (SD) Waist/hip ratio	0.82 (0.06)	0.93 (0.06)	n/a	<.0001	n/a
Median (IQR) systolic BP [mm Hg]	128 (115.0-144.5)	132 (122-141)	123 (113-133)	.57	.42
Median (IQR) diastolic BP [mm Hg]	76 (68-80.5)	78.5 (74-87)	74.5 (63-83)	.22	.69
Mean (SD) creatinine [μmol/L]	74.26 (19.17)	82.83 (35.32)	n/a	.91	n/a
Mean (SD) K ⁺ [mmol/L]	3.89 (0.32)	4.37 (0.59)	n/a	.01	n/a
Median (IQR) onset of diabetes [years]	19.50 (16.00-28.00)	40.00 (32.00-44.00)	n/a	<.0001	n/a
Median (IQR) DM duration [years]	21.38 (8.84-31.00)	6.40 (2.99-14.66)	n/a	.05	n/a
Median (IQR) HbA1c [%]	7.00 (5.90-7.70)	6.90 (6.40-8.10)	n/a	.54	n/a
Median (IQR) FPG [mmol/L]	6.05 (4.85-7.60)	7.90 (6.80-11.55)	4.55 (4.30-5.50)	.03	<.01
Median (IQR) HOMA2-IR	0.95 (0.56-1.28)	2.19 (1.69-2.80)	0.96 (0.77-1.30)	.004	.80
Mean (SD) total cholesterol [mmol/L]	4.30 (0.61)	4.49 (1.15)	4.96 (0.79)	.77	.02
Mean (SD) HDL [mmol/L]	1.59 (0.46)	1.13 (0.30)	1.72 (0.59)	<.01	.55
Insulin treated	8 (44.4%)	7 (38.9%)	n/a	.50	n/a
Statin treated	5 (27.8%)	10 (55.6%)	n/a	.09	n/a
HTN treated	2 (11.1%)	12 (66.7%)	n/a	<.001	n/a

Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; n/a, not applicable; T2DM, type 2 diabetes mellitus.

HNF1A-MODY than in the T2DM group in both genders ([11.3 years vs 3.0 years in men, $P = .008$] and [21.4 years vs 6.4 years in women, respectively, $P = .05$]). Subjects with HNF1A-MODY had significantly lower insulin resistance, measured using HOMA2-IR calculator,²⁴

when compared to BMI-matched individuals with T2DM in both genders (median 0.95 [0.56-1.28] vs 2.19 [1.69-2.80, $P = .004$ in women] and 1.37 [0.64-1.67] vs 1.63 [1.42-2.19], $P = .04$ in men]). There was no difference in insulin resistance between individuals

with HNF1A-MODY and healthy controls in both genders. The number of participants treated with insulin or statins did not significantly differ between HNF1A-MODY and T2DM groups.

There was no significant difference in systolic and diastolic BP in women in all groups but men with HNF1A-MODY had higher systolic BP when compared to healthy controls [median 139 (130.5–148.5) mm Hg vs 125 (117–128) mm Hg, $P = .02$]. There was no difference in systolic BP between both diabetes groups ($P = .39$, Table 1). However, subjects with T2DM were more often treated with anti-hypertensive medications (7/18 men, 12/18 women with T2DM; 2 men and 2 women in HNF1A-MODY group).

4.2 | 11 β -hydroxysteroid dehydrogenase activity

There was no significant difference in the ratio reflecting the activity of 11 β -HSD1 [(THF + 5 α THF)/THE] between groups (Table 2). The activity of 11 β -HSD2 defined by an increased ratio of F/E

and F + E/(5 β THF + 5 α THF+THE) was significantly lower in subjects with HNF1A-MODY when compared to healthy controls in both genders ($P = .001$ and 0.004 in men and $P = .013$ for both in women; Figure 2, Table 2). The activity of both 11 β -hydroxysteroid dehydrogenases was similar in individuals with HNF1A-MODY and T2DM.

There was no gender-related difference in ratios describing the activity of 11 β -HSD2 within each group when examined retrospectively. There was no correlation between ratios defining the activity of 11 β -HSD2 and HbA1c, duration of diabetes, BMI or insulin treatment across all groups examined (all P values nonsignificant).

4.3 | Steroid A-ring reductase activities

Steroid A-ring reductases (5 α - and 5 β -steroid reductase) inactivate cortisol to 5 α -tetrahydrocortisol (5 α THF) and 5 β THF, respectively (Figure 1).

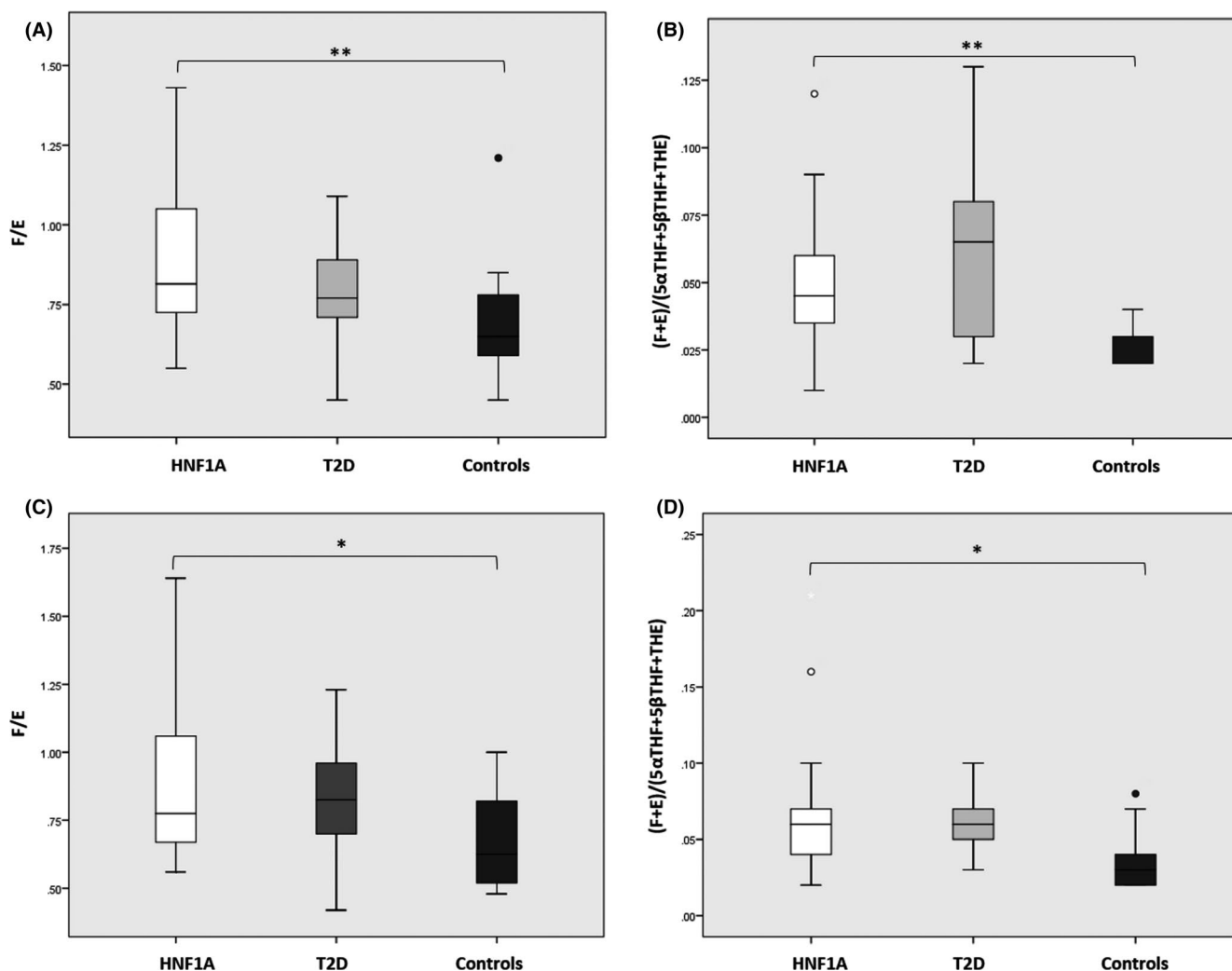


FIGURE 2 Median urine steroid metabolite ratios describing the activity of 11 β -hydroxysteroid dehydrogenase type 2 in men (2A-B) and in women (2C-D) between HNF1A-MODY, T2DM and healthy controls ($n = 16$, 18 and 17 , respectively, in men groups; $n = 18$ in all female groups). * $P < .032$ (threshold of p value significance adjusted for Benjamini-Hochberg procedure), ** $P < .01$ (Mann-Whitney test); F, cortisol; E, cortisone; 5 β THF, 5 β -tetrahydrocortisol; 5 α THF, 5 α -tetrahydrocortisol; THE, tetrahydrocortisone

TABLE 2 Selected urinary steroid metabolites and their ratios in men and women with HNF1A-MODY vs T2DM and healthy controls

Urinary Steroids and their ratios		Median urinary steroid/ ratio (IQR)		P value*		Median urinary steroid/ ratio (IQR)		P value*	
		HNF1A-MODY	T2DM	Control	HNF1A vs T2DM	HNF1A-MODY	T2DM	Control	HNF1A vs T2DM
Gender of the subjects		Men			HNF1A vs CTR	Women			HNF1A vs CTR
Number of subjects		n = 16	n = 18	n = 17		n = 18	n = 18	n = 18	
GC/MC precursor metabolites	Individual steroid metabolites								
	PD (pregnanediol)	191.5 (118.5-310.5)	139.0 (70.0-229.0)	72.0 (56.0-106.0)	.24	165.0 (65.0-409.0)	126.5 (61.0-394.0)	192.0 (109.0-326.0)	.48
	17HP (17-hydroxypregnenolone)	152.5 (93.5-248.0)	99.5 (49.0-138.0)	81.0 (35.0-91.0)	.06	47.5 (18.0-199.0)	22.0 (16.0-60.0)	87.0 (28.0-148.0)	.34
	THS (tetrahydro-11-desoxycortisol)	58.5 (33.0-70.0)	35.0 (12.0-68.0)	28.0 (16.0-40.0)	.16	63.5 (26.0-116.0)	34.5 (23.0-70.0)	33.0 (22.0-43.0)	.41
	5PD (pregnanediol)	461.5 (216.5-600.0)	577.0 (338.0-811.0)	122.0 (65.0-197.0)	.14	129.0 (54.0-447.0)	191.5 (109.0-269.0)	68.5 (43.0-135.0)	.84
GC metabolites	F (cortisol)	131.5 (49.0-196.0)	100.5 (48.0-152.0)	26.0 (19.0-41.0)	.67	126.0 (27.0-189.0)	66.5 (36.0-102.0)	28.5 (20.0-46.0)	.54
	6 β -OH-F (6 β -hydroxycortisol)	161.5 (83.5-277.0)	176.0 (40.0-241.0)	53.0 (40.0-65.0)	.46	225.0 (60.0-394.0)	98.0 (53.0-146.0)	55.5 (46.0-82.0)	.12
	5 β THF (tetrahydrocortisol)	1881.0 (805.5-2209.5)	1218.5 (345.0-2177.0)	749.0 (478.0-1064.0)	.14	1201.5 (382.0-2376.0)	821.5 (420.0-1440.0)	593.0 (415.0-812.0)	.42
	5 α THF (5 α -tetrahydrocortisol)	1054.5 (716.5-1937.0)	1036.0 (633.0-1528.0)	894.0 (530.0-1270.0)	.60	397.5 (152.0-1121.0)	516.0 (323.0-1185.0)	441.5 (237.0-563.0)	.54
	E (cortisone)	144.0 (61.0-215.5)	135.5 (82.0-206.0)	37.0 (32.0-57.0)	.85	154.0 (25.0-229.0)	83.5 (64.0-116.0)	45.0 (31.0-57.0)	.46
MC metabolites	THE (tetrahydrocortisone)	3196.0 (1297.5-3841.0)	2661.0 (978.0-4298.0)	1572.0 (928.0-2531.0)	.46	1687.0 (755.0-3511.0)	1294.0 (672.0-2189.0)	1276.5 (894.0-1628.0)	.48
	THB (tetrahydrocorticosterone)	116.0 (53.5-230.5)	82.5 (22.0-177.0)	46.0 (24.0-88.0)	.28	97.5 (48.0-233.0)	59.5 (33.0-89.0)	46.0 (36.0-63.0)	.09
	TH-DOC (tetrahydro-11-desoxycorticosterone)	14.0 (8.3-21.5)	8.5 (4.0-17.0)	6.0 (5.0-9.0)	.06	12.4 (4.0-35.0)	8.3 (2.0-27.0)	9.5 (6.0-24.0)	.42

(Continues)

TABLE 2 (Continued)

Urinary Steroids and their ratios		Median urinary steroid/ ratio (IQR)		P value*		Median urinary steroid/ ratio (IQR)		P value*	
		HNF1A-MODY	T2DM	Control	HNF1A vs T2DM	HNF1A-MODY	T2DM	Control	HNF1A vs T2DM
Gender of the subjects		Men		Women		Men		Women	
Number of subjects		n = 16	n = 18	n = 17	n = 18	n = 18	n = 18	n = 18	n = 18
Androgen metabolites	Etio (Etiocanolone)	1582.0 (1044.5-3306.0)	1155.0 (703.0-3490.0)	803.0 (641.0-1076.0)	.40	650.5 (528.0-2425.0)	680.5 (452.0-915.0)	753.5 (451.0-1095.0)	.41
	Andro (Androsterone)	2041.0 (1099.5-3118.5)	2249.0 (1175.0-3247.0)	888.0 (755.0-1976.0)	.62	400.5 (268.0-1417.0)	805.5 (389.0-1059.0)	747.0 (508.0-994.0)	.34
Enzyme		Steroid ratios		F/E		F/E		F/E	
11 β -HSD2	(F + E)/ (5 β THF + 5 α THF+THE)	0.82 (0.73-1.05)	0.77 (0.71-0.89)	0.65 (0.59-0.78)	.33	0.77 (0.67-1.06)	0.83 (0.70-0.96)	0.63 (0.52-0.82)	.91
		0.05 (0.04-0.06)	0.07 (0.03-0.08)	0.02 (0.02-0.03)	.33	0.06 (0.04-0.07)	0.06 (0.05-0.07)	0.03 (0.02-0.04)	.72
11 β -HSD1	(5 β THF + 5 α THF)/THE	1.02 (0.85-1.23)	0.87 (0.78-1.24)	1.11 (0.82-1.28)	.25	0.93 (0.76-1.32)	0.97 (0.86-1.24)	0.80 (0.73-0.93)	.70
5 β -steroid reductase	5 β THF/5 α THF	1.56 (0.98-1.84)	0.93 (0.52-1.46)	0.90 (0.67-1.16)	.06	2.20 (1.81-3.78)	1.29 (1.02-2.17)	1.61 (1.24-2.16)	.01
Etio/Andro		0.97 (0.72-1.16)	0.58 (0.45-0.92)	0.87 (0.42-1.15)	.004	1.71 (1.17-2.32)	0.97 (0.74-1.46)	1.17 (0.90-1.49)	.005

Note: Abbreviations: GC, glucocorticoids; MC, mineralocorticoids.

*Significant P value < .032 (in bold) corrected for FDR (false discovery rate) of 10% using Benjamini-Hochberg procedure.

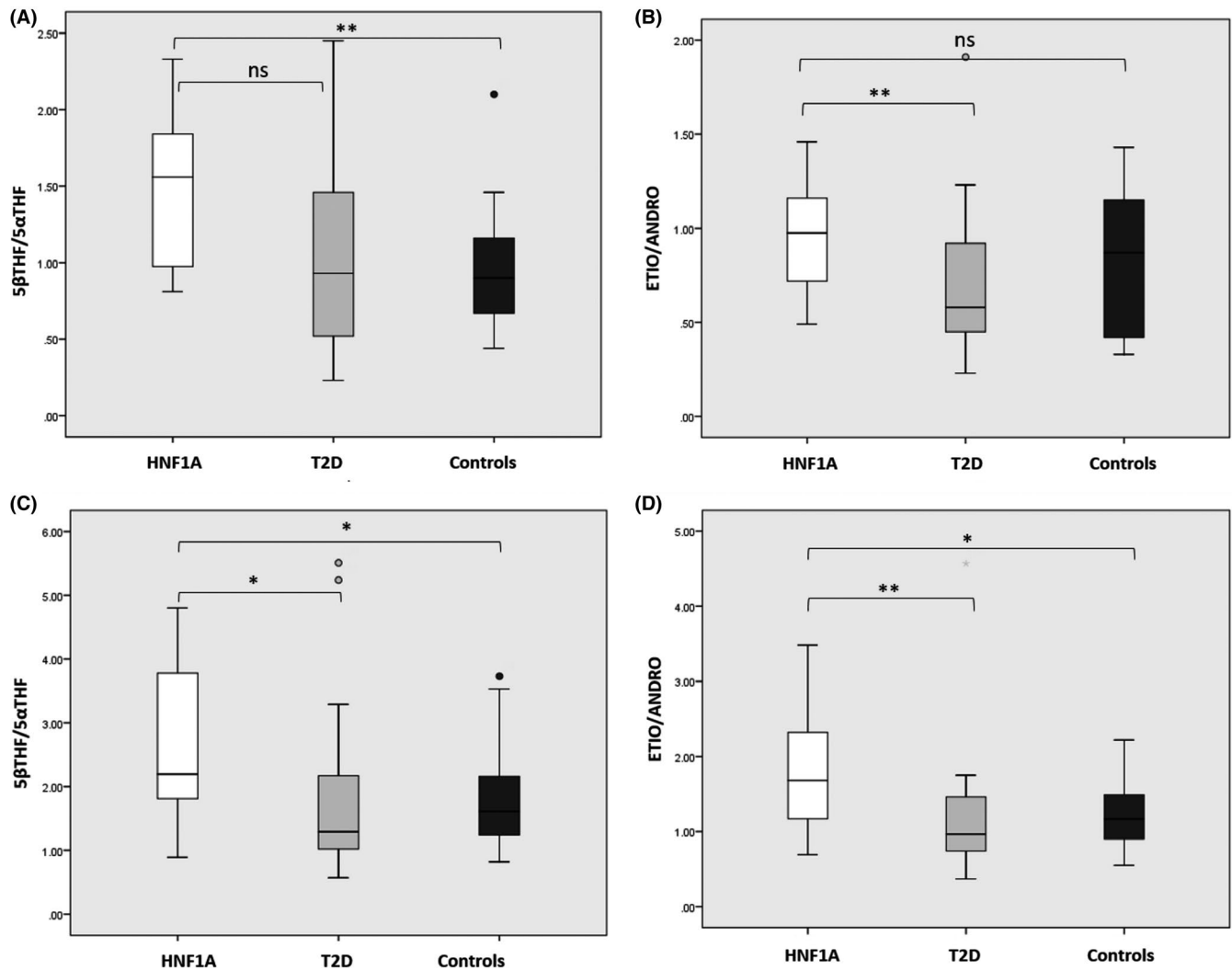


FIGURE 3 Median urine steroid metabolite ratios describing the activity of total 5 α -steroid reductase in men (3A-B) and in women (3C-D) between HNF1A-MODY, T2DM and healthy controls ($n = 16$, 18 and 17 , respectively, in men groups; $n = 18$ in all female groups). * $P < .032$ (threshold of p value significance adjusted for Benjamini-Hochberg procedure), ** $P < .01$; ns, nonsignificant P value (Mann-Whitney test); 5 β THF, 5 β tetrahydrocortisol; 5 α THF, 5 α -tetrahydrocortisol; ETIO, etiocholanolone; ANDRO, androsterone

The ratios of 5 β - to 5 α -metabolites of cortisol (5 β THF/5 α THF and etiocholanolone/androsterone) were higher in subjects with HNF1A-MODY and distinguished them from those with T2DM and healthy controls in women (all $P < .02$, Table 2 and Figure 3). In men, the ratio of 5 β THF/5 α THF was significantly raised in the HNF1A-MODY group when compared with healthy controls ($P = .003$) and the ratio of etiocholanolone/androsterone distinguished men with HNF1A-MODY from those with T2DM ($P = .004$, Figure 3). All median ratios (IQR) and individual p values are provided in Table 2.

There was a sex-related difference in the ratios describing the activity of SRD5A and AKR1D1, which were higher in men than in women in all groups and consistent with previous literature.²⁰ Both ratios defining the activity of SRD5A and AKR1D1 were similar in subjects with T2DM and healthy control groups in both genders.

The ratios of 5 β - to 5 α -metabolites of cortisol reflect the activity of both A-ring reductases. Therefore, an increased ratio of 5 β - to 5 α -metabolites of cortisol reflects a decreased activity of SRD5A or

increased activity of AKR1D1. We cannot definitively unravel the relative contribution of 5 α - and 5 β -steroid reductase in this study, but circumstantial evidence from the known alterations in bile acids in HNF1A-MODY leads us to suspect that this is due to increased AKR1D1 activity (see discussion below).

In women, there was no correlation between ratios describing the activity of AKR1D1 and HbA1c, duration of diabetes, BMI or insulin treatment across all groups (P values nonsignificant). In men, the ratio of etiocholanolone/androsterone correlated with diabetes duration ($r = 0.46$, $P = .007$). There was no correlation with statin or insulin treatment in either gender group.

5 | DISCUSSION

We have identified discrete steroid metabolic pathways that are dysregulated in HNF1A-MODY and T2DM, with both groups

showing evidence of decreased 11 β -HSD2 activity, which would enhance glucocorticoid action. Importantly, HNF1A-MODY patients were distinct in showing an increased 5 β - to 5 α -cortisol metabolites ratio reflecting an increased AKR1D1 activity or decreased SRD5A activity. An increased AKR1D1 activity would result in enhanced glucocorticoid inactivation and partially explain the metabolically favourable phenotype observed in this patient group.

Our data have shown that there is no difference in the global activity of 11 β -HSD1 based on urinary steroid profile in subjects with HNF1A-MODY or T2DM when compared to healthy controls. Increased global activity of 11 β -HSD1 has been suggested to have a role in the pathogenesis of obesity and metabolic syndrome.²⁵ At a tissue-specific level, elevated expression of 11 β -HSD1 in adipose tissue in obese women with IGT and insulin resistance when compared to healthy controls has been reported.^{26–28} Inhibition of 11 β -HSD1 in rodents improves glucose tolerance and insulin sensitivity, making it an attractive antidiabetic treatment target in humans.^{9,10} However, clinical studies have shown only a modest effect on hyperglycaemia.²⁹

We have shown that the activity of 11 β -HSD2 was reduced in subjects with HNF1A-MODY and T2DM when compared to healthy controls in both genders (Table 2, Figure 2). Decreased 11 β -HSD2 activity would be expected to increase local renal cortisol levels and potentially cause local activation of the mineralocorticoid receptor (MR) in a paradigm analogous to that seen in patients with apparent mineralocorticoid excess (AME). AME is caused by genetic defects in HSD11B2 and leads to life-threatening hypertension and hypokalaemia due to aberrant MR activation by cortisol. Circulating cortisol levels are normal as the HPA axis resets to accommodate changes in cortisol half-life and availability. In our study, only men with HNF1A-MODY had significantly higher systolic BP (including 2 individuals on treatment) when compared to age- and BMI-matched healthy controls (139 [130.5–148.5] mm Hg vs 125 [117–128] mm Hg, respectively). There was no difference in BP between HNF1A-MODY and T2DM in both genders in keeping with the fact that the individuals with T2DM expressed similar reduction in the activity of 11 β -HSD2 but they were more often on anti-hypertensive treatment (7 men and 12 women). The changes in 11 β -HSD2 could be important for helping to understand BP regulation and salt and water balance in patients with HNF1A-MODY and T2DM.

Our data demonstrating decreased activity of 11 β -HSD2 differs from a previous report that found no difference in the urinary F/E ratio in obese men with T2DM, although the serum F/E ratio in that group was found to be increased.²⁶ A study comparing BMI-matched individuals with impaired glucose tolerance (IGT) versus healthy participants also showed no significant difference in urinary F/E ratio between groups.²⁷ In both of these studies, participants had a significantly higher BMI (mean 32–34 kg/m²) while our study subjects were leaner (mean BMI 26.5 kg/m²). However, neither cortisol, cortisone nor F/E ratio correlated with BMI in our study.

There should be some caution however in interpreting the activity of HSD11B2 in our participants with diabetes, because the agreement of those ratios between urinary steroids measured in mid-morning urine versus 24-hour urine collection in our method agreement analysis was poor.

We have also found that ratio of 5 β - to 5 α -metabolites of cortisol was increased in subjects with HNF1A-MODY and that this distinguished these individuals from those with T2DM and healthy controls (Table 2, Figure 3). The ratio of etiocholanolone/androsterone was higher in HNF1A-MODY in both men and women, and therefore seems to be a distinctive nonpancreatic feature of HNF1A-MODY.

The 5 β -reductase (AKR1D1) provides an alternative pathway to deactivate cortisol in the liver, but is also an important enzyme in the primary bile acid synthesis pathway.³⁰ Both bile acids and steroid hormones have the potential to regulate the metabolic phenotype.

It is known from both human and animal work that circulating bile acids are increased in HNF1A-MODY subjects when compared to age- and BMI-matched healthy controls.^{16,31} It is plausible that this could be driven by increased activity of AKR1D1 and thus supports our suggestion that the observed elevation of ratio of 5 β - to 5 α -metabolites of cortisol is being driven by the increased activity of AKR1D1 in individuals with HNF1A-MODY.

Our current study also supports previous observations that there is reduced expression of AKR1D1 in T2DM. The expression of AKR1D1 was significantly decreased at both mRNA and protein levels in liver tissue from individuals with T2DM when compared to nondiabetic subjects.³² Accordingly, those with T2DM had reduced hepatic bile acids. This is consistent with our results in women and etiocholanolone/androsterone ratio in men (Figure 3).

Change in bile acids has implications on the composition of the gut microbiota which was also reported to be different in HNF1A-MODY individuals versus healthy controls and subjects with T2DM.³³

6 | CONCLUSIONS

We have reported for the first time the urinary steroid profile in HNF1A-MODY individuals. The activity of two enzymes deactivating cortisol, 11 β -hydroxysteroid dehydrogenase type 2 and steroid 5 β -reductase was significantly altered in subjects with HNF1A-MODY when compared with the healthy controls. The increased ratio of 5 β - to 5 α -steroid metabolites also distinguished subjects with HNF1A-MODY from those with type 2 diabetes. We suggest that this is driven by an increased activity of AKR1D1 in subjects with HNF1A-MODY and may have implications on their phenotype by enhancing tissue glucocorticoid inactivation and altering the bile acid composition and gut microbiota.

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CONFLICT OF INTEREST

No conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Agata Juszcak  <https://orcid.org/0000-0002-1734-6388>

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