Longitudinal changes in glucocorticoid metabolism are associated with later development of adverse metabolic phenotype

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Abstract

Objective: Dysregulation of enzymes that control local tissue steroid metabolism has been implicated in the pathogenesis of obesity and insulin resistance; however, longitudinal changes in glucocorticoid metabolism have not been investigated. This study was performed to evaluate the role of glucocorticoid metabolism in the development of insulin resistance and obesity and to identify biomarkers for future development of metabolic disease.

Design: This was a prospective longitudinal observation study conducted over 5 years.

Methods: A 24-h collection was used to serially analyze urinary glucocorticoid and mineralocorticoid metabolites in 57 obese and overweight patients with no prior diagnosis of diabetes mellitus, recruited from the community.

Results: Baseline higher 5 α -reductase (5 α R) activity, but not 11 β -hydroxysteroid dehydrogenase type 1 activity, was predictive of increased fasting insulin at final visit (11.4 compared with 7.4 mU/l in subjects with lower 5 α R activity, $P < 0.05$), area under the curve insulin response to oral glucose tolerance test (176.7 compared with 89.1 mU/l.h, $P < 0.01$), and homeostasis model assessment (HOMA2-IR; 1.3 compared with 0.8, $P < 0.01$). Higher total glucocorticoid production was associated with abnormal glucose tolerance and increased BMI. During this study, systolic blood pressure increased (equivalent to ~ 1 mmHg/year), as did plasma sodium levels; this evidence of increased mineralocorticoid activity was associated with increased aldosterone metabolites and decreased 11 β -hydroxysteroid dehydrogenase type 2 activity.

Conclusions: Increased 5 α R activity and glucocorticoid secretion rate over time are linked with the development of metabolic disease, and may represent targets for therapeutic intervention, which merits further study.

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Introduction

The worldwide prevalence of obesity is a major public health concern due to its association with increased morbidity and mortality (1). Obesity is a major risk factor for the development of type 2 diabetes mellitus and hypertension (2), although the mechanisms which underpin an individual's progression from normal metabolism through increased insulin resistance and prediabetes to type 2 diabetes remain unclear (3). In addition, insulin resistance is implicated in the development of obesity and

diabetes-associated disease processes such as dyslipidemia and hypertension (4).

Imbalances in steroid hormone metabolism have been postulated as a potential link between insulin resistance and obesity, as well as other components of the metabolic syndrome. This association is most apparent in patients with Cushing's syndrome, in whom elevated circulating cortisol levels drive central adiposity, hyperglycemia, and hypertension (5). In 'simple' obesity, however, circulating

cortisol levels are not elevated, a finding which has led to the exploration of the role of glucocorticoid metabolism in the pathogenesis of metabolic disease (6).

11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) primarily acts to convert inactive cortisone to cortisol, thus amplifying local glucocorticoid action; it is highly expressed in liver, adipose tissue, and muscle (7). 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) is expressed in the kidney and converts cortisol to inactive cortisone; cortisol has a high affinity for the mineralocorticoid receptor (MR) and circulates at much higher concentrations than aldosterone; therefore, 11 β -HSD2 'protects' the MR from being activated by cortisol (8). The glucocorticoids are also metabolized by the A-ring reductases, 5 α - and 5 β -reductases (5 α R and 5 β R), via 3 α -HSD, to their tetrahydrometabolites: 5 α -tetrahydrocortisol (5 α THF), THF, and tetrahydrocortisone (THE). Both 11 β -HSDs and 5 α R have been implicated in the pathogenesis of metabolic disease. 11 β -HSD1 is more highly expressed in adipose tissue from obese individuals and clinical studies of selective 11 β -HSD1 inhibitors have shown improvement in glycemic control, insulin sensitivity, and weight loss (9, 10, 11). The role of the A-ring reductases remains more controversial. Observational clinical studies have identified positive correlation of 5 α R activity with markers of insulin resistance in a variety of clinical cohorts (3, 12), but the impact of therapeutic inhibition remains to be clarified. 11 β -HSD2 has a critical role in the control of blood pressure through regulation of cortisol-mediated activation of the MR. Pharmacological inhibition of 11 β -HSD2 with liquorice (13) or glycyrrhetic acid that leads to hypertension and genetic defects in 11 β -HSD2 cause the syndrome of apparent mineralocorticoid excess (14, 15, 16).

Currently, the significant published data on the role of steroid metabolism in the regulation of metabolic phenotype is cross-sectional; we have therefore conducted the first longitudinal assessment of steroid metabolism to determine prospectively its role in the development of an adverse metabolic phenotype. Specifically, we have aimed to determine whether changes in steroid hormone metabolism (in particular 11 β -HSD1 or 11 β -HSD2, or 5 α R) track with phenotype over a 5-year period, and to determine whether this may act as a predictive biomarker of future disease development.

Subjects and methods

The study was approved by the South Birmingham Local Research Ethics Committee and all subjects gave informed

written consent after full explanation of the purpose and nature of all procedures used (ethics reference 04/Q2707/278). All clinical investigations were carried out at the NIHR/Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital, Birmingham, UK. A total of 57 obese or overweight volunteers (33 females; median BMI 32.8 kg/m², interquartile range (IQR) 30.8–35.0; 47 BMI >30 kg/m²; 56 Caucasian) were recruited into the Birmingham Prospective Obesity Diabetes and Steroid Metabolism (BPODS) cohort following local advertisement and all underwent the clinical protocol. All subjects were invited to return for the same series of investigations yearly for 5 years after the baseline assessment (six visits in total), unless oral glucose tolerance testing revealed diabetes mellitus, in which case they were referred to their general practitioner for appropriate treatment and were withdrawn from the study with development of diabetes as an endpoint. The subjects diagnosed with diabetes mellitus at any time point were included at that time point in analysis of factors contributing to abnormal glucose tolerance (AGT) but did not return for further visits. Not all subjects attended all six visits; therefore, generalized estimating equations were used for analysis of associations rather than repeated measures ANOVA (see Statistical analysis). The subjects had no significant medical history and had normal renal function and blood counts at baseline. None of the subjects had received glucocorticoid therapy (oral, inhaled, or topical) within the 12 months before recruitment. Of the 57 patients, 42 were not on antihypertensives at any time point during the study. Fifteen received antihypertensive therapy at some point during the study; 10/15 received an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker; and no subject received spironolactone or eplerenone during the study.

Clinical protocol

At baseline and at annual follow-up, measurement of BMI, supine waist circumference at the level of the umbilicus, and hip circumference at the level of the greater trochanter was performed. Blood pressure was measured using Dinamap (Critikon, Tampa, FL, USA) while the patient was supine after a 10-min rest, and the average of three readings was recorded.

The subjects fasted for 12 h before each study visit. The blood samples were drawn for measurement of fasting glucose, insulin, HbA1c, total cholesterol, triglycerides, and renal profile. The subjects underwent a 75-g oral glucose tolerance test (OGTT) for 120-min, with blood

sampling being carried out at 30-min intervals for the determination of glucose and insulin levels. The subjects were diagnosed as having AGT if they had one or more of the following diagnoses from the OGTT: impaired fasting glucose, impaired glucose tolerance, or diabetes mellitus, according to the diagnostic criteria established by the American Diabetes Association (17). Fasting glucose and insulin levels were used in the homeostasis model assessment computer model (HOMA2-IR) to generate estimates of insulin sensitivity and β -cell function (HOMA2-IR, HOMA2%S, and HOMA2%B) (18).

Serum and urine analysis

Electrolytes, urea, creatinine, total cholesterol, triglycerides, glucose, and HbA1c were analyzed on a standard automated platform (Roche Modular System, Roche). Insulin was measured using a commercially available colorimetric ELISA (Mercodia, Uppsala, Sweden) with an in-house CV being <5%.

Before attendance at the study center, subjects had a 24-h urine collection for the estimation of corticosteroid metabolites. Urinary corticosteroid metabolite analysis was carried out by gas chromatography/mass spectrometry (GC/MS) as described previously (19); where possible specimens from 2 to 3 years of visits by each patient were analyzed within one batch. Total glucocorticoid metabolites were defined as the sum of cortisol, cortisone, THF, 5 α -THF, THE, α -cortolone, β -cortolone, α -cortol, and β -cortol (20). Urinary cortisol-to-cortisone ratio (F/E) was used as a measure of 11 β -HSD2 activity. The ratio of THF + 5 α -THF/THE and the ratio of cortols/cortolones were used to assess 11 β -HSD1 activity, provided that the F/E ratio was unchanged (21). The activity of 5 α R was assessed using the 5 α -THF/THF ratio (21). The mineralocorticoid metabolites tetrahydroaldosterone (THAldo) and tetrahydrodeoxycorticosterone (THDOC) were also measured as described previously (22).

Statistical analysis

Data analysis was performed using IBM SPSS Statistics (IBM, Armonk, NY, USA). Data were tested for normality using the Shapiro–Wilk test; normally distributed data were described using the mean and s.d., data that were not normally distributed using median and IQR.

In order to test whether any level of markers of steroid metabolism could predict later metabolic phenotype, steroid metabolites and ratios at the baseline visit were dichotomized as greater than and less than the median;

metabolic variables at the last subject visit (year 4 or 5) were compared between the two groups using a Mann–Whitney *U* test. A χ^2 test was performed to assess difference between these groups in those patients who had normal glucose tolerance at baseline and either remained normal or progressed to AGT at some point within the study. For those steroid parameters in which the median was statistically different between male and female subjects, the subjects were designated as ‘greater than’ if the parameter result was greater than the median result for the subject’s gender. Multiple linear regression was then performed to confirm the relationship between metabolic variables and steroid metabolites; age, BMI, gender, 5 α R activity, and total glucocorticoids were included in this analysis.

For the association analysis and effect of time, data that were not normally distributed were log transformed for comparison. The behavior of each continuous variable over time was assessed for linearity before analysis. Of the 57 participants, 23 attended all six scheduled visits. In view of the number of visits missed by study subjects, it was not appropriate to use repeated measures analysis to assess the effect of time in the study; this was performed using generalized estimating equations with a first-order autoregressive correlation structure. The use of generalized estimating equations allowed data from all 57 participants to be analyzed without imputation because the data did not have to be complete for each participant (as would be the case for repeated measures ANOVA); and allows the inclusion in the model of time-dependent covariates (rather than just their baseline values). The associations were modeled graphically and presented in the figures. Significance was accepted at a *P* value of <0.05.

Results

The number of subjects attending each year and their clinical characteristics are given in Table 1. At baseline, median (IQR) BMI was 32.8 kg/m² (30.8, 35.0); systolic BP (SBP) was 126 mmHg (115, 142) and diastolic BP (DBP) 76 mmHg (64, 84). Baseline clinical characteristics in those who did not attend all six visits or were withdrawn from the study were not significantly different from those of the 23 subjects who attended all six return visits (data not shown).

Baseline urinary total 24-h glucocorticoid metabolites were higher in men than in women (14 303 (11 312, 17 371) vs 7537 (5476, 9603) μ g/24 h, *P*<0.001), as were F/E (0.65 (0.61, 0.77) vs 0.50 (0.45, 0.59), *P*<0.001) and 5 α THF/THF (0.94 (0.72, 1.50) vs 0.58 (0.45, 1.00), *P*=0.02) ratios.

Table 1 Clinical data from Birmingham Prospective Obesity Diabetes and Steroid Metabolism (BPODS) cohort. Data presented are medians and interquartile ranges in parentheses.

	Baseline (n=57)	Year 1 (n=54)	Year 2 (n=43)	Year 3 (n=46)	Year 4 (n=31)	Year 5 (n=38)
Age (years)	52 (46, 57)	53 (48, 58)	54 (48, 59)	55 (49, 60)	56 (48, 62)	57 (52, 62)
Gender (F:M)	33:24	30:24	23:20	29:17	18:13	24:14
BMI (kg/m ²)	32.8 (30.8, 35.0)	33.5 (30.9, 35.8)	32.5 (29.8, 35.8)	32.4 (30.2, 36.4)	34.3 (30.9, 36.8)	33.1 (29.7, 37.4)
SBP (mmHg)	126 (115, 142)	131 (121, 139)	132 (118, 142)	133 (120, 144)	135 (118, 142)	136 (126, 146)
DBP (mmHg)	76 (64, 84)	77 (70, 85)	74 (70, 81)	76 (68, 86)	75 (68, 85)	78 (69, 86)
GT status (AGT, %)	22 (39%)	13 (24%)	12 (28%)	10 (22%)	10 (32%)	9 (24%)
Na (mmol/l)	140 (138, 142)	140 (139, 142)	141 (140, 143)	142 (140, 143)	142 (141, 143)	142 (140, 145)
Antihypertensive therapy	15 (26%)	15 (28%)	12 (28%)	9 (20%)	6 (19%)	8 (21%)

F:M, female:male; SBP, systolic blood pressure; DBP, diastolic blood pressure; GT, glucose tolerance status (AGT, abnormal glucose tolerance includes impaired fasting glucose, impaired glucose tolerance, and diabetes mellitus); Na, plasma sodium; antihypertensive therapy, number of subjects taking an antihypertensive at that visit. n=number of subjects who attended each visit.

Urinary steroid metabolites as a predictor of the development of metabolic phenotype

Baseline urinary total 24-h glucocorticoid metabolites, 5α THF+THF/THE, 5α THF/THF, and F/E ratios were grouped into quantiles of greater than or less than the median adjusted for gender; differences in metabolic variables between groups were then examined using data from the final visit (year 4 or 5).

The subjects with higher 5α R activity at baseline had significantly increased fasting insulin (11.4 (8.6, 15.7) vs 7.4 (3.2, 12.5) mU/l, $P<0.05$) and area under the curve for insulin across an OGTT (AUC; 176.7 (96.0, 244.1) vs 89.1 (65.0, 140.4) mU/l.h, $P<0.01$) at final visit when compared with those with lower baseline 5α R activity (Fig. 1a and b). Higher 5α R activity was also predictive of higher final HOMA2-IR (1.3 (0.9, 1.8) vs 0.8 (0.4, 1.4), $P<0.01$) (Fig. 1c), higher HOMA2%B (113 (96, 138) vs 91 (53, 121)%, $P<0.05$), and lower HOMA2%S (77 (57, 112) vs 123 (71, 256)%, $P<0.01$). Multiple linear regression analysis confirmed the predictive analysis (Table 2). There was no effect of age, BMI, or gender in these models. Baseline 5α R activity did not predict final visit AUC glucose (Fig. 1d).

Total glucocorticoid metabolites were assessed for predicting the value of final visit triglyceride levels of $>$ or <1.7 mmol/l, a level that forms part of the diagnosis of metabolic syndrome, but this approach failed to identify any predictive marker. Regression analysis, however, identified baseline visit total glucocorticoid metabolites to be associated with higher final visit triglycerides (Table 2). Change from normal glucose tolerance at baseline to AGT at any time point in the study and final visit SBP were not predicted by baseline steroid parameters. Importantly, baseline urinary 5α THF+THF/THE or F/E ratios were not predictive of final visit insulin, HOMA measurements, or any other measured metabolic variable.

Prospective longitudinal changes in glucocorticoid metabolism and metabolic phenotype

In the 57 patients, log total glucocorticoid metabolites decreased during the study (coefficient -0.019 , $P=0.002$, 95% CI -0.031 to -0.007).

Log 5α -THF/THF, reflecting 5α R activity, increased during the study (coefficient 0.020 , $P=0.002$, 95% CI 0.012 to 0.028) and was higher in men than in women. 5α R activity was not related to age at study entry. Log 5α -THF+THF/THE and log cortols/cortolones (reflecting 11β -HSD1 activity) did not change during the study.

Figure 1

Baseline 5α R activity measured by urinary 5α THF/THF ratio (divided into high or low reflecting values above or below median ratio adjusted for gender) predicts final visit fasting insulin (a), AUC insulin across oral glucose tolerance test (b), and HOMA2-IR (c); but do not predict AUC glucose across oral glucose tolerance test (d) (* $P<0.05$, ** $P<0.01$).

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Table 2 Multiple linear regression models for (A) final visit fasting insulin (B), final visit area under the curve insulin on oral glucose tolerance test (C), final visit HOMA2-IR, and (D) final visit triglycerides. Total glucocorticoids divided by 1000 so that the coefficients are of the same order of magnitude as those for the other variables. A total of 43 participants included in model. Coefficient for effect of sex in model is for female participant.

Model	Parameter	Coefficient	95% CI	P value
(A) Fasting insulin RSq=0.28	Intercept	−8.719	−33.681, 16.242	0.484
	Age	−0.043	−0.332, 0.246	0.766
	Sex (F)	−0.121	−2.802, 2.559	0.928
	BMI	0.429	−0.098, 0.957	0.108
	Total glucocorticoids	0.118	−0.297, 0.533	0.568
	5 α THF/THF	6.431	1.525, 11.336	0.012
(B) Insulin AUC RSq=0.14	Intercept	28.158	−324.177, 380.494	0.872
	Age	0.143	−3.937, 4.223	0.944
	Sex (F)	1.171	−36.249, 38.592	0.949
	BMI	1.168	−6.803, 9.139	0.768
	Total glucocorticoids	0.453	−5.352, 6.257	0.875
	5 α THF/THF	72.695	4.040, 141.349	0.039
(C) HOMA2-IR RSq=0.27	Intercept	−1.028	−3.798, 1.742	0.457
	Age	−0.005	−0.037, 0.028	0.775
	Sex (F)	−0.025	−0.322, 0.273	0.868
	BMI	0.051	−0.008, 0.109	0.087
	Total glucocorticoids	0.012	−0.034, 0.058	0.606
	5 α THF/THF	0.678	0.134, 1.222	0.016
(D) Triglycerides RSq=0.55	Intercept	1.182	−0.654, 3.019	0.199
	Age	−0.008	−0.031, 0.014	0.442
	Sex (F)	−0.188	−0.381, 0.006	0.058
	BMI	−0.000	−0.037, 0.037	0.990
	Total glucocorticoids	0.044	0.015, 0.074	0.005
	5 α THF/THF	0.071	−0.291, 0.432	0.694

5 α THF/THF, 5 α reductase activity.

Increasing BMI was associated with higher log total glucocorticoid metabolites, reflecting daily cortisol secretion rate (coefficient 0.013, $P < 0.001$, 95% CI 0.006 to 0.020) and log 5 α THF/THF (coefficient 0.008, $P = 0.02$, 95% CI 0.001 to 0.015) but not 5 α THF+THF/THE ratios. There was an effect of gender (Fig. 2) but not waist-hip ratio (data not shown). Modeled associations are presented in Fig. 2a, b, and c.

Deteriorating glucose tolerance status was associated with higher log total glucocorticoid metabolites (coefficient 0.074, $P = 0.001$, 95% CI 0.030 to 0.118), but not with log 5 α THF/THF or log 5 α THF+THF/THE ratios. Female subjects were more likely than male subjects to have persistently normal glucose tolerance on serial measures (19/33 vs 5/24, Fisher's exact test, $P = 0.007$).

Prospective longitudinal changes in blood pressure and mineralocorticoid activity

In the 57 patients, log SBP increased during the study (coefficient 0.005, $P = 0.01$, 95% CI 0.001 to 0.008) (Fig. 3a) equating to approximately an increase of 1 mmHg in SBP

per year. The increase in log DBP failed to reach significance ($P = 0.07$). BMI was associated with increasing log SBP (coefficient 0.002, $P = 0.02$, 95% CI 0.000 to 0.003). However, age at entry into the study ($P = 0.17$), AGT status ($P = 0.4$), and gender ($P = 0.1$) showed no association with blood pressure.

Across the duration of the study, logTHAldo (coefficient 0.025 95% CI 0.010 to 0.040, $P < 0.001$) and logF/E (coefficient 0.012 95% CI 0.004 to 0.020, $P = 0.003$) increased, whilst logTHDOC (coefficient −0.025 95% CI −0.043 to −0.007, $P = 0.006$) decreased (Fig. 3b, c, and d). These observations were adjusted for BMI and glucose tolerance status. In addition, log serum sodium increased (coefficient 0.001 95% CI 0.001 to 0.002, $P < 0.001$) (Fig. 3e). Log serum potassium did not change (data not shown).

The observations were similar in the 42 antihypertensive treatment-naïve subjects when compared with the complete cohort; both SBP and DBP increased during the study and this increase was associated with increased urinary THAldo. The association of time in the study and logTHAldo, logTHDOC, and logF/E ratio was independent of gender and BMI.

Figure 2

Modeled association between total urinary cortisol metabolites and BMI for men and women (a); between urinary 5αTHF/THF ratio (5αR activity) and BMI for men and women (b); and between urinary 5αTHF+THF/THF ratio (11β-HSD1 activity) and BMI for men and women (c). *P* values quoted are for difference between men and women. As BMI increases, both total glucocorticoids and 5αR activity increase (*P* value < 0.001 for both measures of steroid metabolites). No significant change (NS) in 11β-HSD1 activity with BMI change, nor significant difference between men and women. White squares, men; black squares, women.

Discussion

In this prospective, longitudinal study, we have characterized corticosteroid metabolism, both as a predictor of the future development of metabolic phenotype and also as a direct contributor to the mechanisms underpinning disease.

5αR activity was higher in men than in women and higher levels predicted the development of metabolic phenotype (fasting insulin, AUC insulin, insulin sensitivity, and insulin secretion) at a visit 4 or 5 years later; as such it may represent a potential 'biomarker' for future development of metabolic disease. Previously published data have shown that increased 5αR activity is associated with an adverse metabolic phenotype, including insulin resistance and hepatic steatosis (3, 12, 23); however, our study represents the first longitudinal analysis. The functional significance of these observations has not been fully determined; however, increased 5αR activity will increase glucocorticoid clearance and this may act as a protective mechanism to reduce tissue exposure to glucocorticoids (24). It is also possible that this may simply be a reflection of hyperinsulinemia as 5αR activity and expression are stimulated by insulin (25). Finally, increased 5αR activity may drive androgen excess that could fuel insulin resistance (26). Although this was a longitudinal study, it remains to be clarified whether increased 5αR activity represents a cause or consequence of metabolic disease. We have previously shown that 5αR activity decreases with weight loss (27, 28), and emerging evidence from rodent models and clinical studies suggest that 5αR knockout or inhibition is detrimental to metabolic health. These observations would suggest that increased 5αR activity is a protective mechanism in the setting of adverse phenotype. It was not possible to split the subjects into quartiles of baseline 5αR activity because of the sample size of the study, but inclusion of 5αR in the models for metabolic outcomes (Table 2) confirmed the relationship between 5αR and fasting insulin, insulin AUC, and HOMA2-IR. 5αR activity did not predict final visit AUC glucose; this reflects the length of the study and the fact that impaired glucose tolerance occurs at a later point than insulin resistance in the development of type 2 diabetes mellitus.

In contrast to the clinical significance of 5αR activity, there is clear evidence from *in vitro* and animal studies that increased 11β-HSD1 activity has a pathogenic role in the development of metabolic disease (29, 30, 31, 32). Genetic manipulation and pharmacological inhibition improve blood pressure, lipid profiles, and insulin resistance in

Figure 3

Modeled association between blood pressure and time (a). White circles, systolic; black circles, diastolic. In both men and women, logTHAldo increased with time (b); whilst logTHDOC decreased (c). LogF/E increased with time, consistent with decreased 11β -HSD2 activity (d). White squares, men; black

animal models (9, 33), and clinical studies using selective 11β -HSD1 inhibitors have now shown improvement in glycemic control in patients with type 2 diabetes mellitus, as well as reductions in BP and weight (10, 11, 34). However, in this study, 11β -HSD1 activity did not predict development of metabolic disease. This does not lessen its potential as a therapeutic target, but suggests that analysis of basal 11β -HSD1 activity by urinary steroid metabolite measurement, which is a measure of global 11β -HSD1 activity and cannot identify organ-specific responses to metabolic change, may not identify those patients who are likely to respond to selective 11β -HSD1 inhibitors.

Total glucocorticoid production was persistently higher in male than in female subjects, and was higher in subjects with AGT compared with those with a normal OGTT (NGT); a relationship we have described previously in female subjects (3). Total glucocorticoid production at baseline was not predictive of final visit glucose tolerance status, but this probably reflects the fact that the change in glucose tolerance status in individuals was not linear, i.e. each patient did not progress inexorably from NGT to

squares, women. *P* values shown are for effect of time. For all three steroid parameters, there was a difference between men and women: log THAldo $P<0.05$; logTHDOC $P<0.01$, log F/E $P<0.001$. Furthermore, serum Na increased with time (e) consistent with enhanced MR activation.

prediabetes and then diabetes (clearly illustrated in Table 1). This may reflect weight loss in individuals who did not impact on the overall increase in BMI in the group over time. Over time in the group as a whole, higher

Figure 4

The changing pattern of adrenal corticosteroid metabolism with time that contributes to the development of an adverse metabolic phenotype. MR, mineralocorticoid receptor.

glucocorticoid production was associated with increasing BMI and worsening glucose tolerance. The mechanisms by which this might contribute to dysglycemia and adiposity are not clear. Increased adrenal glucocorticoid output is offset by increased metabolism to maintain circulatory levels, but tissue-specific responses to glucocorticoid turnover may be important and we were not able to assess this using urinary metabolite measurement. For example, 11 β -HSD1 activity in the liver may decrease to restore circulatory levels, but this may not occur in skeletal muscle or adipose tissue; this may be important in driving adverse metabolic phenotype including dysglycemia and increased fat mass.

Systolic blood pressure increased in this cohort over the 5-year duration of the study and whilst this was independent of age, it was associated with increasing BMI. Our prospective analysis has allowed the identification of two different mechanisms that may contribute to this; first, increased aldosterone production (reflected in THAldo levels) and secondly, reduced 11 β -HSD2 activity (increased F/E ratio), both of which will lead to enhanced MR activation.

The increase in THAldo and decrease in THDOC, a metabolite of deoxycorticosterone, suggest increased activity of aldosterone synthase. This is unlikely to be related to increased stimulation by ACTH, as total glucocorticoid production rates decreased over the duration of the study, and serum potassium levels were unchanged (data not shown). The association with BMI is intriguing. Angiotensinogen production by adipose tissue has been reported and it is possible that this may contribute to aldosterone synthesis in obese subjects (35), although we were not able to test this hypothesis in this cohort.

The increase in urinary F/E ratio over the duration of the study is an indication of reduced 11 β -HSD2 activity. Cortisol circulates in higher concentrations than aldosterone and has the same affinity for the MR; receptor selectivity for aldosterone is conferred by 11 β -HSD2, which converts active cortisol to inactive cortisone (8). Our observations would therefore suggest that increased MR exposure to cortisol driving salt and water retention contributes to increased BP over time in our obese cohort. A previous cross-sectional study has suggested that aging was associated with reduced 11 β -HSD2 activity (36); that study showed an increase in cortisol and decrease in cortisone in the older subjects, as measured by an isolated serum F/E ratio. It is difficult to interpret F/E in serum but our use of 24-h urine steroid analysis provided a more

robust assessment of corticosteroid metabolites over a 24-h period (37).

It is unclear from our findings whether aldosterone or reduced 11 β -HSD2 activity is the predominant factor in the rise in SBP observed. In other cases of hypertension mediated by low 11 β -HSD2 activity, or inhibition, plasma renin falls with consequent fall in aldosterone (13). In this study, we did not collect plasma for renin measurement and so cannot comment on the impact of reduced 11 β -HSD2 activity on the renin–angiotensin–aldosterone system; regardless, one or both of these mechanisms have led to a rise in sodium in this cohort.

In summary, in this study, we have conducted the first longitudinal analysis of corticosteroid secretion and metabolism and its relationship with metabolic phenotype. We have proposed that over time, increased production of both glucocorticoids and mineralocorticoids as well as tissue-specific changes in corticosteroid metabolism contribute to an adverse metabolic phenotype (Fig. 4). This has identified potential biomarkers for the development of disease and possible underlying mechanisms that may have implications for targeted therapeutic intervention.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

R K Crowley analyzed the data and wrote the manuscript, B Hughes measured and analyzed the urinary steroid metabolites, J Gray and T McCarthy collected the clinical data, S Hughes analyzed the insulin samples, C H L Shackleton analyzed the urinary steroid metabolites and provided control material, N Crabtree contributed to the design of the study, P Nightingale designed the statistical analysis plan for the study and contributed to analysis of the data, P M Stewart contributed to the design of the study and analysis of the data, and J W Tomlinson designed the study, analyzed the data and wrote the manuscript. All authors contributed to the final draft of the manuscript.

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