Steroids 74 (2009) 742–745

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Urinary free cortisone, but not cortisol, is associated with urine volume in severe obesity

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ARTICLE INFO

Article history:
Received 22 December 2008
Received in revised form 24 February 2009
Accepted 23 March 2009
Available online 2 April 2009

Keywords:
UFF
UFE
Tetrahydrocortisol
5α-Tetrahydrocortisol
Tetrahydrocortisone
11β-Hydroxysteroid dehydrogenase type 2

ABSTRACT

Background: High urine volume enhances urinary free cortisol (UFF) and cortisone (UFE) excretion rates in normal-weight adults and children. Renal excretion rates of glucocorticoids (GC) and their metabolites are frequently altered in obesity. The aim of the present study was to investigate whether UFF and UFE excretion is also affected by urine volume in severely obese subjects.
Experimental: In 24-h urine samples of 59 extremely obese subjects (mean BMI 45.3 ± 8.9 kg/m^2) and 20 healthy lean subjects (BMI 22.1 ± 1.8 kg/m^2), UFF and UFE, tetrahydrocortisol (THF), 5α-tetrahydrocortisol (5α-THF), and tetrahydrocortisone (THE) were quantified by RIA. The sum of THF, 5α-THF, and THE (GC3), the three major GC metabolites, reflects daily cortisol secretion. 11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity was assessed by the ratio UFE/UFF. Daily GC excretion rates were corrected for urine creatinine and adjusted for gender and body weight.
Results: In extremely obese subjects, urine volume was significantly associated with creatinine-corrected UFE and 11β-HSD2 activity after adjustment for gender and BMI ($r = 0.47$, $p = 0.0002$ and $r = 0.31$, $p = 0.02$, respectively). However, urine volume was not associated with creatinine-corrected UFF and GC3 ($p = 0.4$ and $p = 0.6$, respectively). In lean controls, urine volume was significantly associated with creatinine-corrected UFE and UFF ($r = 0.58$, $p = 0.01$ and $r = 0.55$, $p = 0.02$, respectively), whereas urine volume was not associated with 11β-HSD2 activity after appropriate adjustment ($p = 0.3$).
Conclusions: In severe obesity, in contrast to normal weight, renal excretion of UFE, but not of UFF is affected by fluid intake. This discrepancy may be due to the increased renal 11β-HSD2 activity in obesity.

1. Introduction

Measurement of the urinary excretion of glucocorticoids (GCs) and their metabolites provides a non-invasive, integrated evaluation of adrenocortical GC secretion and the potentially biological active free GCs, urinary free cortisol (UFF), and cortisone (UFE) over a period of 24 h in health and disease [1]. UFF frequently serves as a single index of adrenocortical function and is the most common screening test for endogenous hypercortisolism [2]. Previous studies indicate UFE as a valuable complementary analyte to UFF for a more comprehensive evaluation of functional adrenal activity [1,3,4]. Combined measurement of UFF and UFE may help in the diagnosis of factitious Cushing's syndrome following hydrocortisone ingestion [5]. Furthermore, the ratio of UFE to UFF is an established measure for the assessment of renal 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts cortisol to inactive cortisone [6,7]. Recent studies in normal-weight adults and children showed that a high fluid intake and a corresponding high urine volume increased renal excretion rates of UFF and UFE [8–11]. Thus, urine volume appears to be an important confounding factor in the adrenocortical evaluation based on renal GC excretion.

In obesity, cortisol metabolism and correspondingly renal GC metabolite excretion rates are frequently altered [12]. Furthermore, activities of enzymes involved in pre-receptor metabolism of GCs,

Abbreviations: BP, blood pressure; BMI, body mass index; E, cortisone; F, cortisol; GC, glucocorticoid; GFR, glomerular filtration rate; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; 11β-HSD2, 11β-hydroxysteroid dehydrogenase 2; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; MDRD, Modification of Diet in Renal Disease; OGTT, oral glucose tolerance test; RIA, radioimmunoassays; THE, tetrahydrocortisone; THF, tetrahydrocortisol; 5α-THF, 5α-tetrahydrocortisol; TSH, thyroid-stimulating hormone; UFE, urinary free cortisone; UFF, urinary free cortisol.

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0039-128X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2009.03.007

such as 11β-HSD2 and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which reactivates cortisone to active cortisol, appear to be tissue-specifically influenced in obesity [13]. In clinical practice, measurement of UFF, to test for endogenous hypercortisolism, is an integral part of the endocrine work-up of obesity [2]. However, the influence of water load on renal UFF and UFE excretion has, so far, not been systematically studied in obesity. Therefore, the aim of the present study was to investigate the association between urine volume and renal GC excretion in severely obese subjects.

2. Experimental

2.1. Patients and recruitment

Recruitment to the study commenced in July 2005, and the study was completed in October 2008. Patients were recruited from the obesity outpatient clinics at the University Hospital of Tübingen. Inclusion criteria were age from 18 to 65 years and obesity (body mass index (BMI) >30 kg/m^2). Exclusion criteria were major comorbidity, comprising severe hypertension (systolic blood pressure ≥180 mmHg and/or diastolic blood pressure ≥120 mmHg), endocrine disorders, including diabetes mellitus and hypothyroidism, and chronic renal failure as assessed by a glomerular filtration rate (GFR) <90 ml/min/1.73 m^2. 20 Healthy, lean subjects served as controls.

2.2. Metabolic characterization

An oral glucose tolerance test (OGTT) was performed as formerly described in detail [14]. Plasma glucose and insulin concentrations were measured as recently described [14]. Insulin sensitivity from the OGTT (in arbitrary units) was calculated as reported earlier [14].

Serum and urine creatinine were measured enzymatically using an automated analysis system (ADVIA 1650 Chemistry Systems; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA).

2.3. GC measurements

UFF, UFE, tetrahydrocortisone (THE), tetrahydrocortisol (THF), and 5α-tetrahydrocortisol (5α-THF) were measured by in-house radioimmunoassays (RIA) at the Steroid Laboratory of the Department of Pharmacology, University of Heidelberg, using tritiated steroids (Amersham Pharmacia Biotech, Freiburg, Germany) and antisera, raised and characterized in the steroid lab as described elsewhere [15]. Cross-reactivities were as follows: for cortisol antiserum to cortisone; 0.5%; to corticosterone, 5.6%; to 11-deoxycortisol, 48.3%; to 11-deoxycorticosterone, 0.5%; to 17-hydroxyprogesterone, 2.6%; to 21-deoxycortisol 60.4% and to THE, THF, 5α-THF, <0.2%; for cortisone antiserum to cortisol, 2.9%; to corticosterone, 0.9%; to 11-deoxycortisol, 25.1%; to 11-deoxycorticosterone, 1.3%; to THE, 2.6%; to THF and 5α-THF, 0.2%; for THE antiserum to cortisol, 0.2%; to cortisone, 0.3%; to THF, 9.7%; to 5α-THF, 0.1%; for THF antiserum to cortisol, cortisone and corticosterone, <0.1%; to THE, 0.3%; to 5α-THF, <0.1%; for 5α-THF antiserum to cortisol and cortisone, <0.1; to THF, 0.7; to THE, <0.1. Before RIA we performed recovery-corrected extraction and chromatographic purification for UFE and UFF, thereby efficiently removing cross-reacting steroids. In more detail, steroids were extracted with dichloromethane and chromatographically separated as previously described with modifications [16]. Celite (Celite 545 AW; Sigma Aldrich Chemie, Steinheim, Germany) was used as an inert support for partition chromatography. The stationary phase consisted of ethylene glycol; while the mobile phase consisted of a mixture of dichloromethane in isooctane with increasing polarity eluting cortisone with 80% dichloromethane and cortisol subsequently with 60% ethyl acetate in isooctane. THE, THF, and 5α-THF were quantified in a final dilution of 1:1200 (vol:vol) as described elsewhere [17,18] with some modifications. Before RIA, urine samples were hydrolysed with β-glucuronidase from Escherichia coli K12 (Roche Diagnostics, Germany) for 12 h at 37 °C to get total (conjugated plus unconjugated) tetrahydrometabolites of cortisol and cortisone. Analytical sensitivity of the assays was for UFE and UFF, 1.5 μg/l; for THE, 0.08 mg/l; for THF and 5α-THF, 0.06 mg/l. Analytical recovery (spiked samples, mean ± SD, $n = 9$) and linearity recovery (samples serially diluted 2–32-fold) were for UFF, 108.0 ± 4.0%, and 101.7 ± 13.4%; for UFE, 101.7 ± 5.4%, and 96.5 ± 7.2%; for THE, 100.6 ± 10.7%, and 96.2 ± 5.2%; for THF, 106.7 ± 8.3%, and 99.4 ± 7.9%; for 5α-THF, 100.7 ± 6.8%, and 98.8 ± 5.6%. Intra- and interassay coefficients of variation were for all assays <10% and <15%, respectively.

2.4. Calculations and assessments

24-h excretion rates of the major urinary GC metabolites THE, THF, and 5α-THF were summed, to assess the adrenal gland's daily GC secretion [19,20]. Enzyme activity of 11β-HSD2, which transiently inactivates cortisol to cortisone, was assessed by the ratio UFE/UFF.

2.5. Estimation of glomerular filtration rate (GFR)

To asses renal function in our study subjects GFR was estimated from plasma creatinine using the abbreviated MDRD (Modification of Diet in Renal Disease) formula [21]:

$$\mathrm{GFR\,(ml/min/1.73\,m^2)} = 0.186\,(\mathrm{plasma\ creatinine}) - 1.154 \times (\mathrm{age}) - 0.203 \times (0.742\ \mathrm{if\ female}) \times (1.210\ \mathrm{if\ African\text{-}American}).$$

2.6. Data analysis

Unless otherwise stated data are given as mean ± standard deviation (SD). Distribution was tested for normality using the Shapiro–Wilk W test. Non-normally distributed parameters were ln-transformed prior to statistical analysis. Multivariate linear regression analysis was performed after adjustment for potential confounding variables, using the least squares method. A p-value <0.05 was considered statistically significant. The statistical software package JMP 7.0 (SAS Institute, Cary, NC, USA) was used.

Daily GC excretion rates were corrected for urine creatinine and adjusted for gender and body weight as follows: each individually calculated 24-h analyte/creatinine ratio was multiplied by individual body weight and by a gender-specific factor (male: $y = 1.75 + 0.006 \times$ deviation from ideal body weight [%]; female: $y = 1.1 + 0.0045 \times$ deviation from ideal body weight [%]; [22]). Ideal body weight was assessed in males as (height [cm] − 100) × 0.9 and in females as (height [cm] − 100) × 0.85.

2.7. Ethical approval

The local ethics committee had approved the protocol (protocol number 350/2007B01). Informed written consent was obtained from all participants.

3. Results

3.1. Characterization of study population

Subject characteristics are summarized in Table 1. The mean age of the 59 non-diabetic, obese subjects was 40 ± 13 years. Given