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Intraindividual stability of cortisol and cortisone and the ratio of cortisol to cortisone in saliva, urine and hair

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ABSTRACT

Background: Cortisol, cortisone and the ratio of cortisol to cortisone in saliva, urine and hair are acute, short-term and long-term biomarkers to reliably assess the activity of hypothalamic-pituitaryadrenal (HPA) axis and 11β-hydroxysteroid dehydrogenase (11β-HSD). One key issue is whether these biomarkers have intraindividual relative stability. Salivary, urinary and hair cortisol was proven to show considerable long-term intraindividual relative stability. However, currently unknown is whether cortisone and the ratio in saliva, urine and hair show intraindividual relative stability.

Methods: The present study utilized a longitudinal design to validate long-term stability within two weeks of three biomarkers in saliva and urine, and long-term stability within twelve months of three hair biomarkers. Salivary, urinary and hair steroids were measured with high performance liquid chromatography tandem mass spectrometry.

Results: Three biomarkers in urine and hair showed moderate test-retest correlations with coefficient (*r*) ranging between 0.22 and 0.56 and good multiple-test consistencies with coefficient of intraclass correlation (*ICC*) ranging between 0.42 and 0.67. Three single-point salivary biomarkers showed weak to moderate test-retest correlations (*r's* between 0.01 and 0.38) and poor to fair multiple-test consistencies (*ICC's* between 0.29 and 0.53) within two weeks. Three single-day salivary biomarkers showed moderate test-retest correlations (*r's* between 0.23 and 0.53) and good multiple-test consistencies (*ICC's* between 0.56 and 0.66) within two weeks.

Conclusions: Three biomarkers in urine and hair showed moderate long-term intraindividual relative stability. Three single-point salivary biomarkers showed weak to moderate short-term and long-term intraindividual relative stability, but three single-day salivary biomarkers showed moderate short-term and long-term intraindividual relative stability.

1. Introduction

Endogenous cortisol in biomatrices (e.g., blood, saliva, urine and hair) has been proven to be a reliable biomarker reflecting the activity of the hypothalamic-pituitaryadrenal (HPA) axis [1]. Endogenous cortisone in biomatrices is regarded to be an alternative biomarker of cortisol [2–5]. The resulting ratio of cortisol to cortisone (R_{cc}) is the potential biomarker to reliably assess the activity of 11β-hydroxysteriod dehydrogenases (11β-HSD) where 11β-HSD type 1 (11β-HSD_1) is responsible for the reversible conversion of cortisone to cortisol and 11β-HSD type 2 (11β-HSD_2) catalyzes the irreversible conversion of cortisol to cortisone [6,7].

The three biomarkers have been widely applied to physical and mental health field, such as obesity [8], metabolic syndrome [2], major depression [9], bipolar disorder and schizophrenia [10], chronic fatigue syndrome [11,12] and post-trauma stress disorder [13]. In these studies, the biomarkers are used to assess the functional activities in a relatively long time. Thus there is implicit assumption underlying the use of the biomarkers that in the absence of external factors the biomarkers show a high level of intraindividual stability including absolute stability and relative stability (i.e., test-retest relative stability and multiple-test consistency), at least show a high level of intraindividual relative stability. Given the importance of this assumption, previous studies had examined the

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patterns of intraindividual stability of cortisol in blood, saliva, urine and hair [14–30]. It was found that cortisol in various biomatrices have no absolute stability [20,27]. However, the results on the relative stability varied with studies. For instance, previous studies demonstrated that hair cortisol shows weak to strong test-retest relative stability and multiple-test consistency where there is weak to high test-retest Pearson's correlation or intraclass correlation of multiple tests [17,19,21,26–28]. The reasons for the difference in the relative stability might be mainly attributed to the differences in the trait of the population and time interval period from several days to years. Additionally, temporal characteristics of cortisol vary with the biomatrices. Single-point levels in blood and saliva reflect acute level from several minutes to several hours, single-day levels in blood, saliva and urine (e.g., area under the circadian rhythm curve, AUC) reflect the accumulative levels from several hours to one day and multiple-day levels in blood, saliva and urine and hair level reflect the accumulative levels from several months to years [31]. Therefore it is necessary to simultaneously validate intraindividual stability of cortisol in different biomatrices among the identical population. Additionally, the intraindividual stability of cortisone level and R_{cc} remains unclear.

On the other hand, previous studies used R_{cc} value in saliva and urine (R_{scc} and R_{ucc}) to mainly reflect local activity of 11β-HSD_2 in salivary glands and kidney rather than the overall activity of 11β-HSD isozymes [6], and R_{cc} value in hair (R_{hcc}) to represent the integrated activity of 11β-HSD isozymes in various peripheral organs and tissues because endogenous cortisol and cortisone in hair are thought to mostly result from active or passive diffusions of free blood-related species [32] and hair matrix records the accumulative levels of blood-borne cortisol and cortisone [33]. Moreover, single-point salivary level is representative of acute biomarker, and single-day level in saliva and urine is representative of short-term biomarker and hair level is representative of long-term biomarker. Therefore, the present study focused on intraindividual stability within two weeks of the three biomarkers in saliva and urine, and intraindividual stability within twelve months of hair biomarkers.

Based on the literature discussed above, it was hypothesized that cortisol in saliva, urine and hair might have no absolute stability and show considerable intraindividual relative stability. Similar results would be true for cortisone and R_{cc} in saliva, urine and hair.

2. Materials and methods

2.1. Study design and participants

The present study conducted two longitudinal designs to test the above hypotheses in which saliva and urine samples were collected three times within a two-week period with an interval of one week and hair samples were collected three times within a twelve-month period with an interval of six months.

Ninety-five non-obese healthy college students (male: 51, female: 44; age mean: 18.72 ± 0.75 Y, range: 17–21 Y; BMI mean: 21.62 ± 3.04 kg/m^2, range: 17.57–27.57 kg/m^2) recruited from an agriculture university in Nanjing, China. Of these, 79 participants provided their hair samples and frequency of hair washing per week (mean: 3.42 ± 1.48 time/week, range: 1–7 time/week). Exclusion criteria were the same as described previously [34]. All participants provided written informed consent before inclusion. This study followed the Declaration of Helsinki and was approved by the health Science Research Ethics Board of Southeast University.

2.2. Collection of saliva, urine and hair samples

All participants provided 12 copies of saliva samples and 3 copies of overnight urine samples on three different weekends (D_1, D_2 and D_3) with an interval of one week. Amongst them, 79 participants provided 3 copies of hair samples in three different months (M_1, M_2 and M_3) with an interval of six months.

Saliva samples were collected in the awakening time (T_1: about 7:30–8:15 am), early morning (T_2: 8:30 am), noon (T_3: 12:00 am) and afternoon (T_4: 5:00 pm), and overnight urine (10:00 pm-7:30 am) was taken in the next morning. Participants were asked to collect their saliva and urine samples by themselves at their dormitory in the weekend. Prior to saliva collection, participants were asked to gargle with water and collected saliva with 4-mL plastic tube 5 min after gargling. Additionally, participants were asked to refrain from brushing their teeth, smoking, eating and drinking prior to saliva collection. Participants were asked to collect overnight urine with 10-mL plastic tube in the morning. Prior to urine collection, participants were asked to refrain from eating and drinking after 10:00 pm last night. As-collected saliva and urine samples were frozen at −50 °C until analyzed.

Hair samples with longer than 1 cm in the posterior vertex region were cut with iron scissors as close as possible to the scalp. As-collected hair strands were stored in dry tubes at −20 °C for the analysis of cortisol and cortisone. The hair samples were cut as 1-cm hair segments prior to analysis. The 1-cm hair segment closest to the scalp was used to mark steroids' status during the past 1-month period.

2.3. Analysis of salivary, urinary and hair cortisol and cortisone

Detection of steroids was done in a 3200 QTRAP high-performance liquid chromatography tandem mass spectrometer (ABI, USA) equipped with atmospheric pressure chemical ionization sources (LC-APCI-MS/MS). Steroids were measured in positive mode as previously described [35]. The LC-APCI-MS/MS method showed the limit of detection at 0.05 ng/ml for salivary steroids and 0.1 ng/ml urinary steroids and 0.5 pg/mg for hair steroids. Intra- and inter-day precisions were less than 10% at standard concentration of 2, 10 and 200 ng/ml for salivary and urinary steroids (or 5, 25 and 50 pg/mg for hair steroids) and recovery ranged between 95% and 105% at the three concentrations.

2.4. Statistical methods

Statistical analyses were performed using SPSS 20.0 for Windows. Variables that were non-normally distributed were logarithmically transformed for next statistical analysis. Pearson's correlation analysis was conducted to examine test-retest relative stability in the three biomarkers where coefficient of Pearson's correlation (r) <0.30 means weak stability and $0.30 \leqslant r < 0.60$ means moderate stability and $r \geqslant 0.60$ means high stability. Intraclass correlation using a two factor mixed effect model was to examine consistency among multiple measures at four different time points or three different collection days for the three biomarkers where coefficient of the intraclass correlation (ICC) <0.40 means poor consistency and $0.40 \leqslant ICC < 0.60$ means fair consistency and $0.60 \leqslant ICC < 0.75$ means good consistency and $0.75 \leqslant ICC < 1.00$ means excellent consistency. Repeated measures analysis of variance with Greenhouse-Geisser correction was conducted to examine absolute stability across different times in the three biomarkers where $p > 0.05$ means that there is absolute stability and $p < 0.05$ means no absolute stability.

3. Results

3.1. Stability of three salivary biomarkers

The single-point levels of salivary cortisol, cortisone and R_{scc} varied with time in the daytime on the three collection days