Association of Obesity with Cortisol Levels in Saliva, Urine and Hair Among Male College Students

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Abstract. Pathologically cortisol exposure is closely related to obesity. However, relevant results were inconsistent because previous studies utilized different inclusion criteria, and different cortisol biomarker, such as salivary, urinary and hair cortisol. This study performed an observational case-control study to explore association of obesity with cortisol through comparing the differences in levels of salivary, urinary and hair cortisol between the 25 obese male college students and 25 age-matched normal students. The results revealed that obese students had a strong trend to be higher hair cortisol levels than controls, but there were no differences in salivary and urinary cortisol levels between them. Our results suggest that hair cortisol rather than salivary and urine cortisol levels might be a sensitive and reliable tool to assess the long-term activity of the hypothalamus-pituitary-adrenal axis in the obese students.

Introduction

Obesity is a global pandemic with major health consequences at a level of an individual as well as a public health [1, 2]. Given the occurrence of Cushing’s syndrome with abdominal obesity and high cortisol level, previous studies focused on whether cortisol plays a role in the expression of obesity in the general population. However, previous relevant results were inconsistent with studies showing positive, negative association and no association [2]. The reasons might be attributed to variations of inclusion criteria and biomarker with studies. Firstly, the previous inclusion criteria used different anthropometric parameters, such as body mass index (BMI), or waist circumference (WC) or waist-to-hip ratio (WHR). Secondly, in the previous, basal cortisol activity was measured with different biomarkers, such as salivary, urinary, and hair cortisol. These biomarkers showed different temporal characteristics, for example, single-point salivary cortisol is the acute biomarker, single-day salivary and urinary cortisol are the short-term biomarker, multiple-time average of salivary and urinary cortisol and hair cortisol are the long-term biomarker [2]. Considering obesity is a disorder in a relatively long time, there is a temporal mismatch between cortisol assessments and obesity when acute and short-term biomarkers were used to assess the activity of hypothalamic-pituitary-adrenal axis. Therefore, it is necessary to utilize long-term biomarkers of cortisol and inclusion criteria combined with different anthropometric parameters for examining association of cortisol with obesity among the identical population.
The present study used the inclusion criteria combined with BMI and WC and multiple-day levels of salivary and urinary cortisol and hair cortisol as cortisol biomarker. It aimed to explore association of long-term cortisol level with obesity among college students through examining whether there is significant difference in cortisol level between the obese students and normal controls.

Subjects and Methods

Subjects

This cross-sectional case-control study recruited two groups (the obese and normal groups) aged between 18 and 21 years from a university hospital in Nanjing, China. All participants received oral and written information about the study. Exclusion criteria were the same as described previously [3]. Finally, the obese group consisted of 25 male college students with overall obesity and abdominal obesity (BMI≥28 kg/m² and WC≥85 cm) and the normal group consisted of 25 male college students without abdominal obesity (18.5<BMI<24 kg/m² and WC<80 cm). The study was conducted according to the Declaration of Helsinki and was approved by the health Science Research Ethics Board of Southeast University.

Anthropometric Parameters

Body weight and height were measured, and BMI was calculated. WC was measured halfway between the lower rib and the iliac crest using a non-elastic flexible tape measure with the subject standing without clothes covering the waist area. Obesity and abdominal obesity was defined according to The Working Group on Obesity in China cutoff values for BMI and WC (WGOC, 2002) [1].

In order to exclude students with the metabolic syndrome in the obese group, total, high-density lipoprotein- and low-density lipoprotein-cholesterol (TL-c, HDL-c and LDL-c) and triglycerides (TG) and fasting plasma glucose (FPG) in a blood sample and blood pressure (systolic blood pressure, SBP; diastolic blood pressure, DBP) and heart rate (HR) were measured in hospital. There were no obese students with the metabolic syndrome based on their biological parameters (SBP: 131.40±8.14 mmHg; DBP: 76.84±8.99 mmHg; HR: 82.32±9.47 beats/min; FPG: 5.29±0.45 mmol/L; TL-c: 5.13±0.89 mmol/L; HDL-c: 1.36±0.47 mmol/L; LDL-c: 2.72±0.55 mmol/L; TG: 1.28±0.37 mmol/L).

Saliva, Urine and Hair Collection

All participants provided 12 copies of saliva samples and 3 copies of overnight urine samples on three different weekends (D₁, D₂ and D₃) with an interval of one week, and 1 copy hair samples was collected two weeks later after the collection of saliva and urine in order to well match the time span of salivary and urinary collections. The detail of samples collection described elsewhere [3].

Analysis of Cortisol and Cortisone in Saliva, Urine and Hair

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). The purification of salivary steroids was done according to the procedures described elsewhere [4-6]. 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 steroids concentrations.

Statistical Analysis

Statistical analyses were performed using SPSS 20.0 for Windows (IBM SPSS Statistics). The normally distributed data are presented as $M \pm SD$ where $M$ and $SD$ are mean and standard deviation, and the non-normally distributed data were presented as median and range and were logarithmically transformed for next statistical analysis. The area under the curve (AUC) was used to assess the single-day output of salivary cortisol. One-way analysis of variance (ANOVA) was conducted for comparison between groups. An analysis of covariance (ANCOVA) was conducted for salivary and urinary cortisol when age was used as a covariate and for hair cortisol with age and hair washing frequency (HWF) as covariates.

Results

As listed in Table 1, the obese students showed significantly higher weight, BMI and WC than the normal ones ($p<0.001$), but there were no significant differences in age and HWF ($p>0.05$).

| Table 1. Comparison in age, weight, BMI, WC and HWF between the obese and normal students. |
|---------------------------------------------|-----------------|-----------------|-----------------|
| Age, [y]                                   | Obesity         | Normal          | Statistical value |
| Body weight, [kg]                          | 18.75±0.57      | 18.86±0.64      | $F_{(1,48)}=0.397, p=0.532$ |
| BMI, [kg/m²]                               | 93.08±8.82      | 60.40±3.57      | $F_{(1,48)}=294.502, p<0.001$ |
| WC, [cm]                                   | 30.17±2.35      | 20.31±1.23      | $F_{(1,48)}=344.455, p<0.001$ |
| HWF, [time/week]                           | 99.22±9.92      | 72.90±3.86      | $F_{(1,48)}=152.809, p<0.001$ |
|                                           | 4.18±2.10       | 4.10±1.76       | $F_{(1,48)}=0.021, p=0.885$ |

As listed in Table 2, there was no difference in urinary cortisol level between obese and normal groups on all three collection days ($p>0.10$). The differences remained insignificant ($p>0.10$) after age being as a covariate. However, the obese students showed a significantly higher salivary cortisol level than controls on D2 ($p<0.05$), but showed a strong trend to be significantly lower salivary cortisol level on D3 ($p=0.085$), and no difference on D1 ($p=0.195$). After age being as a covariate, the difference remained insignificant on D1 ($p>0.10$), but the difference became marginally significant on D2 ($P=0.089$) and insignificant on D3 ($P=0.300$).

As listed in Table 2, there were no significant differences between obese and normal subjects in three-time average levels of salivary and urinary cortisol ($p>0.10$). The differences remained insignificant ($p>0.10$) after age being a covariate. Notably, the obese students showed a strong trend to be significantly higher hair cortisol level than controls ($p=0.077$), but the difference became insignificant ($p=0.205$) after age and HWF being covariates.
Table 2. Differences in salivary, urinary and hair cortisol level between the obese and normal subjects.

<table>
<thead>
<tr>
<th>Cortisol Levels</th>
<th>Obesity</th>
<th>Normal group</th>
<th>Statistical value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary cortisol [ng/ml]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>59.18,19.04-118.79</td>
<td>47.99,19.04-179.54</td>
<td>$F_{(1,48)}=1.728, p=0.195$</td>
</tr>
<tr>
<td>D2</td>
<td>57.09,13.58-94.75</td>
<td>39.31,8.56-123.42</td>
<td>$F_{(1,48)}=4.125, p=0.028$</td>
</tr>
<tr>
<td>D3</td>
<td>49.36,12.92-193.08</td>
<td>59.03,14.83-348.00</td>
<td>$F_{(1,48)}=3.097, p=0.085$</td>
</tr>
<tr>
<td>Three-time average</td>
<td>55.64,24.61-122.71</td>
<td>48.68,27.49-196.54</td>
<td>$F_{(1,48)}=0.023, p=0.879$</td>
</tr>
<tr>
<td>Urinary cortisol [ng/ml]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>109.24,8.08-330.32</td>
<td>108.05,7.15-442.68</td>
<td>$F_{(1,48)}=0.283, p=0.597$</td>
</tr>
<tr>
<td>D2</td>
<td>81.31,14.78-284.42</td>
<td>102.92,13.84-303.81</td>
<td>$F_{(1,48)}=1.414, p=0.240$</td>
</tr>
<tr>
<td>D3</td>
<td>94.65,21.45-232.44</td>
<td>90.83,7.53-376.52</td>
<td>$F_{(1,48)}=0.025, p=0.875$</td>
</tr>
<tr>
<td>Three-time average</td>
<td>104.31,31.63-199.72</td>
<td>115.75,56.73-257.40</td>
<td>$F_{(1,48)}=1.242, p=0.271$</td>
</tr>
<tr>
<td>Hair cortisol [pg/mg]</td>
<td>9.36,2.21-31.40</td>
<td>6.90,0.82-23.16</td>
<td>$F_{(1,48)}=3.275, p=0.077$</td>
</tr>
</tbody>
</table>

**Discussions**

The current study found that obese students showed a strong trend to be higher hair cortisol levels than normal controls, but showed no different levels of salivary and urinary cortisol from controls. To the best of our knowledge, this is the first study to simultaneously investigate long-term cortisol level in saliva, urine and hair among obese and normal subjects.

Regarding the association of hair cortisol levels with obesity, the present finding in early adults was generally consistent with previous results [7-9]. Wester et al found that obese adult patients had significantly higher hair cortisol levels than normal subjects [7]. Veldhorst et al found that obese children had a trend to be significantly higher hair cortisol levels than normal subjects when the ethnicity being a covariate [8]. Chan et al found that obese adults had higher hair cortisol levels than non-obese adults, but the difference was insignificant [9]. The inconsistency might be because 75 % of obese subjects fulfilled diagnosis criteria of MetS were included in the study of Wester et al [7] and 15 % of obese subjects fulfilled diagnosis criteria of MetS were included in the study of Veldhorst et al [8] and the non-obese group included overweight and normal subjects in the study of Chan et al [9].

This study also found that obese subjects had no significantly higher short-term and long-term urinary cortisol levels, and long-term salivary cortisol levels than controls, but showed significantly higher or lower short-term salivary cortisol levels. The similar results were observed in previous studies showing no significant differences in salivary and urinary cortisol levels [9-11], or significantly lower salivary cortisol levels [12] and higher urinary cortisol levels in obese subjects [13, 14]. The observation might be because cortisol levels in saliva and urine are characterized by marked circadian rhythm [15] and day-to-day variability for an identical individual [16, 17]. In particular, salivary cortisol levels are strongly influenced by specimen collection, physiological and psychological stressors and urinary cortisol levels are strongly influenced by water loading [18] and sport training [19].

Notably, obese students had a strong trend to be higher hair cortisol levels than normal students, but showed no difference in salivary and urinary cortisol levels between them. This might be because cortisol in hair is mostly originated from the active or passive diffusions of free cortisol in blood that is trapped and gradually deposited in the growing hair shaft [20]. Hair cortisol retrospectively records cumulative cortisol reflecting long-term basal activity of the HPA.
system. In contrast, cortisol in saliva and urine only record cortisol level reflecting acute and short-term activity of the HPA system. The current findings suggested that hair cortisol rather than salivary and urinary cortisol levels is a useful tool to measure long-term cortisol exposure in individuals and to assess the long-term activity of the HPA axis in obese individuals.

There are some limitations in the current study. Firstly, this study was limited to salivary and urinary cortisol levels in three separate days rather than multiple consecutive days. Previous studies suggested that multiple sampling in consecutive days might be stable for representing the long-term cortisol levels [21]. Secondly, this study was limited to a small-size sample with single-gender and with narrow age range participants. Homogenous sample with a small size might bring an adverse effect on the extension of our results. Thirdly, our study did not investigate demographic information and some life events, such as socioeconomic status, perceived stress [22]. Future studies with larger sample sizes are needed to evaluate the effect of perceived stress, socioeconomic status on cortisol levels and obesity.

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References


