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Short communication

Underweight subjects with anorexia nervosa have an enhanced salivary cortisol response not seen in weight restored subjects with anorexia nervosa

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ABSTRACT

The cortisol response to awakening (CAR) has been reported to be enhanced in symptomatic patients with anorexia nervosa (AN). However, it has been not established whether the dysregulation of CAR was a primary phenomenon or a change secondary to malnutrition. Therefore, we aimed to explore the salivary CAR in both underweight and weigh-restored women with AN. Fifty-nine women volunteered for the study. They were 18 underweight AN women, 15 weight-restored AN women and 26 normal-weight healthy women. Saliva samples were collected in the morning, immediately on awakening and after 15, 30 and 60 min to measure saliva levels of cortisol. Participants' anxiety levels in the morning of sampling were measured by the State-Trait Anxiety Inventory. As compared to control women, underweight AN patients showed an enhanced CAR whereas weight-restored patients had a normal CAR. These results could be not explained by group differences in body mass index or levels of anxiety. These findings show, for the first time, that the enhanced CAR occurring in the acute phase of AN is not seen in weight-recovered patients, suggesting that the dysregulated activity of the hypothalamus-pituitary-adrenal axis of symptomatic AN patients is a state-dependent phenomenon.

1. Introduction

Anorexia nervosa (AN) is a psychiatric disorder characterized by prolonged periods of starvation with or without occasional binge episodes followed by purging and/or other compensatory behaviors aiming to prevent body weight (BW) gain. Dysregulated eating leads to chronic malnutrition, which may be responsible for several physical complications, including endocrine alterations such as hyperactivity of the hypothalamus-pituitary-adrenal (HPA) axis, which is likely due to an overdrive of the corticotrophin-releasing factor (CRF)/adrenocorticotropic hormone (ACTH) system (Lo Sauro et al., 2008). The HPA axis is the main effector system of the body stress response, and an association between stressful life events and the onset or the maintenance of AN has been documented (Pike et al., 2006; Rojo et al., 2006). Furthermore, evidence has been provided that CRF has anorexic, anxiogenic and depressogenic properties (Hillebrand et al., 2002), therefore, in underweight AN patients CRF hypersecretion, although secondary to malnutrition, might contribute to their aberrant heating behaviour and/or their anxiety and depressive symptoms.

In the last decade, the cortisol awakening response (CAR), that is the immediate rise in cortisol secretion after awakening, has received a growing attention as a reliable indicator of the HPA axis activity and has been believed to provide useful information on the HPA axis functioning that cannot be derived from other cortisol measures (Stalder et al., 2016). We recently reported an increased CAR in adult patients with symptomatic AN, but we could not establish whether the enhanced CAR was a primary phenomenon or a change secondary to malnutrition because of the lack of a control group of weight-restored AN patients (Monteleone et al., 2011, 2014, 2015). To our knowledge, studies on CAR in weight-recovered AN patients have not been performed so far. Therefore, we explored the CAR in both undernourished and weight-restored AN patients to test the hypothesis that, if malnutrition is

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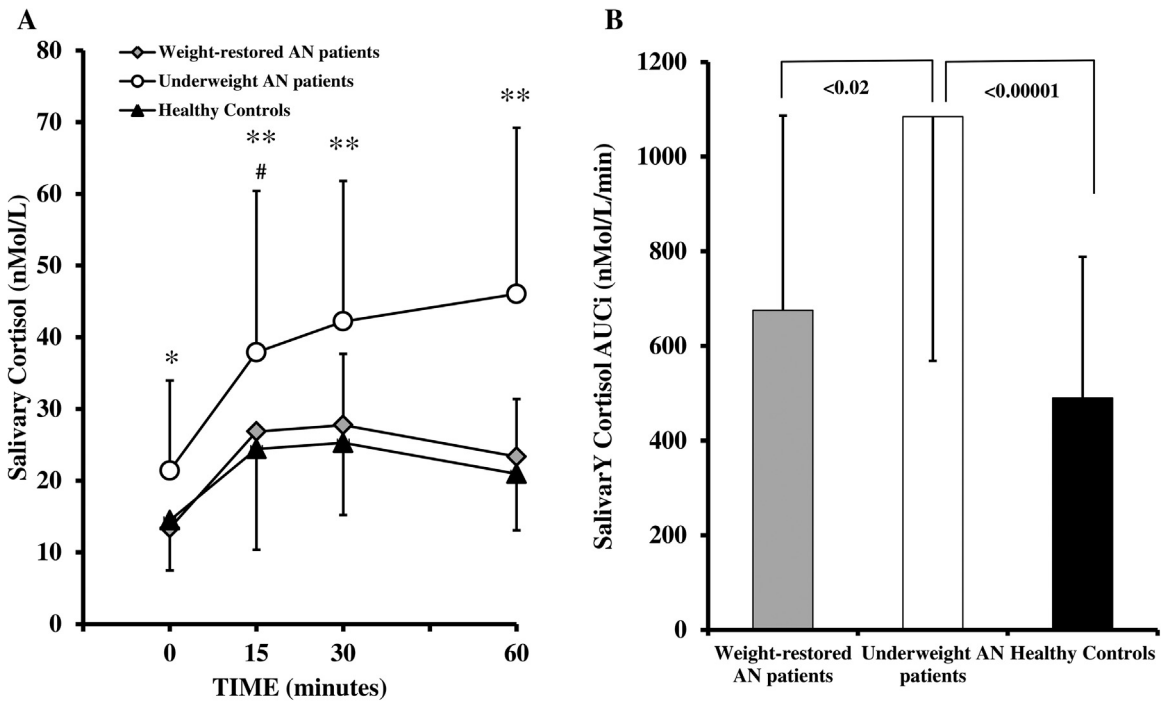

Fig. 1. Salivary cortisol response to awakening (panel A) and area under the curve with respect to the increase (AUCi) (panel B) in underweight patients with anorexia nervosa (AN), weight-restored patients with AN and healthy controls. Data are expressed as mean ± SD. *P < 0.02; **P < 0.001 vs both healthy controls and weight-restored AN patients (post hoc Tukey's test) #P < 0.005 vs weight-restored AN patients (post hoc Tukey's test).

responsible of increased CAR in underweight patients with AN, this increase should be absent in weight-restored AN patients.

2. Material and methods

Fifty-nine women volunteered for this study. They were 33 patients recruited from those consecutively admitted to the outpatient unit of the Eating Disorder Center of the Department of Psychiatry of the University of Naples SUN, according to the following inclusion/exclusion criteria: (a) diagnosis of AN, either present or past, according to DSM-IV criteria; (b) age ≥18 yrs; (c) absence of severe physical disorders or comorbid psychiatric disorders; (d) no history of psychosis, endocrine disorders, psychoactive substance use or head trauma; (e) no use of drugs in the past 2 months. Diagnoses were made by using the Structured Clinical Interview for DSM-IV Axis I disorders-patient edition (SCID-I) (First et al., 1995). Eighteen underweight female patients were diagnosed with current AN and 15 normal weight female patients were diagnosed with past AN, since they had a restored normal body mass index (BMI), i.e., BMI ≥ 18.5 kgm^{-2}. Eating-related psychopathology was assessed by the Eating Disorder Inventory-2 (EDI-2) (Garner, 1991). Underweight patients were tested before starting any specific weight-restoring program; weight-restored patients were tested 4–14 weeks after reaching the normal BMI; all of them had recovered their menstrual cycle.

Twenty-six healthy women were included as the control group. They were drug-free and mentally healthy, as assessed by SCID-I non-patient edition (First et al., 1996). Control women and weight-restored AN patients were tested in the follicular phase of their menstrual cycle.

The experimental protocol was approved by the local ethics committee and all subjects gave their written consent after being fully informed of the nature and procedures of the study.

Participants were instructed to collect saliva samples at home immediately on awakening and 15, 30 and 60 min post-awakening. During this time they had to refrain from eating, drinking (except water), smoking and brushing teeth, to fill in the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1983) and to record the times when they went to sleep and when they woke up. Saliva samples were collected into salivette tubes (Sarstedt; Rommelsdorft, Germany) that were stored in home freezers before being returned to the lab, where cortisol levels were measured by an enzyme immunoassay method, using a commercially available ELISA kit (Biochem Immunosystem, Milan, Italy). Intra- and inter-assay coefficients of variation were less than 8% and 8.7%, respectively. In order to maximize the participants' adherence to the sampling procedure, as suggested by Stalder et al. (2016), we tried to motivate them in an initial face-to-face meeting where they not only were instructed about the sampling procedure but were also engaged with research goals. Moreover, they were provided with take-home written instructions and, whenever possible, a relative supervised the whole sampling procedure.

The CAR was calculated also as the cortisol area under the curve with respect to the increase (AUCi). The BMDP statistical software package (Dixon, 1985) was used for data analysis. A two-way analysis of variance (ANOVA) with repeated measures followed by the post-hoc Tukey's test was employed to test differences in CAR among the groups. Differences in saliva cortisol AUCi, demographic, clinical and anthropometric variables among groups were tested by one-way ANOVA. Since groups significantly differed in current BMI, state and trait anxiety scores, these variables were introduced as covariate in the ANOVA.

3. Results

3.1. Clinical and demographic data

Participants' clinical and demographic characteristics are shown in Table 1.

No significant differences emerged between patients and controls in the mean time of awakening as well as in the mean duration of sleep the night before saliva sampling. Both underweight and