

Original Article

The Circadian Variation of Cortisol Secretion in Patients with Anorexia Nervosa in Childhood and Adolescence after Recovery of Body Weight by Treatment Using Gas Chromatography/Mass Spectrometry in Selected Ion Monitoring

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Abstract. Anorexia nervosa (AN) is a chronic psychiatric disorder which is characterized by patient-induced weight loss. Complications in many organ systems can be seen in AN such as cardiovascular, gastrointestinal, and endocrine system including hypothalamic-pituitary-adrenal axis, even after recovery of body weight by treatment. Urinary steroid profile analysis using gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring (SIM) has been reported to be useful for the diagnosis of abnormal steroidogenesis in newborn infants, childhood, and adults. The aim of this study was to analyze the circadian variation of cortisol secretion in patients with anorexia nervosa (AN) in childhood and adolescence after recovery of body weight by treatment using GC/MS in SIM. The subjects were 7 healthy young adults (20–23 yr of age, BMI 19.7–24.8 kg/m²) and 5 AN patients in childhood and adolescence (13–19 yr of age), who had recovered body weight by treatment (BMI 15.4–19.3 kg/m²; 3rd–25th to 50th percentile). Urine samples were collected for 26 hours (from 21:00 to 23:00 next day) at each urination. In each sample, the cortisol metabolites were measured by GC/MS in SIM. The sum of all cortisol metabolites was calculated as mg/g creatinine. In all 5 AN patients in childhood and adolescence, the circadian variation of the sum of cortisol metabolites was observed and was similar to that in healthy young adults. Although our data are preliminary, in patients with AN in childhood and adolescence, who have recovered body weight by treatment, the circadian variation of cortisol secretion may be conserved.

Key words: anorexia nervosa, GC/MS-SIM, childhood, adolescence, circadian variation, cortisol

Introduction

Anorexia nervosa (AN) is a chronic psychiatric disorder which is characterized by patient-induced weight loss. Recently AN has been an important health concern among childhood and adolescence in advanced countries. AN in childhood and adolescence can have

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Table 1 Five AN patients undergoing treatment

subject number	at underweight					at study				
	age yr	height cm	weight kg	BMI kg/m ²	percentile	age yr	height cm	weight kg	BMI kg/m ²	percentile
1	14.8	154.1	34.2	14.4	< 3 rd	19.7	155.0	46.3	19.3	10 th to 25 th
2	15.2	154.3	35.7	15.0	< 3 rd	18.7	158.8	45.4	18.0	3 rd to 10 th
3	12.4	139.7	18.5	9.5	< 3 rd	13.4	146.2	38.0	17.8	25 th to 50 th
4	16.1	158.7	38.7	15.4	< 3 rd	16.5	158.8	43.1	17.1	3 rd
5	12.9	159.8	39.3	15.4	3 rd to 10 th	13.4	161.0	47.6	18.4	25 th to 50 th

At underweight indicates the lowest BMI percentile calculated by available past height and weight measurements (13). At study indicates the day of urine sample collection.

medical complications in many organ systems such as cardiovascular, gastrointestinal, and the endocrine system, which might persist even after recovery of body weight. A number of endocrine systems can be abnormal including for example appetite-regulating peptides, monoaminergic systems, the hypothalamic-pituitary-adrenal axis, the hypothalamic-pituitary-ovarian axis, the hypothalamic-growth hormone -insulin-like growth factor I (IGF-I) axis, the hypothalamic-pituitary-thyroid axis, and the bone metabolism (1–5).

Urinary steroid profile analysis using gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring (SIM) has been reported to be useful in the diagnosis of abnormal steroidogenesis in newborn infants, childhood, and adults (6–8).

An altered circadian variation of cortisol secretion is assumed to be characteristic for some chronic psychiatric disorders (9, 10). Therefore, the aim of this study was to analyze circadian variation of cortisol secretion using the sum of cortisol metabolites in urine by GC/MIS in SIM in patients with AN in childhood and adolescence after recovery of body weight by treatment.

Subjects and Methods

The subjects were 7 healthy young adults

(20–23 yr of age, 6 female and 1 male, BMI 19.7–24.8 kg/m²) and 5 AN patients undergoing treatment in childhood and adolescence (13–19 yr of age, all female). Diagnosis of AN was based on the diagnostic criteria for AN in childhood and adolescence (11). All patients had been treated by the “Keio Method” (comprehensive multi-modal program focused on retrieval of healthy adolescent growth and development) for more than one year (12). The BMI of the patients were 15.4–19.3 kg/m²; 3rd–25th to 50th percentile (Table 1) (13). All subjects gave their informed consent for this study.

The subjects urinated ad lib. Urine samples were collected for 26 h (from 21:00 to 23:00 next day) at each urination to investigate the circadian variation of cortisol secretion. Each sample was kept at –20°C until analyzed. In each sample, the cortisol metabolites were measured (5 β -tetrahydrocortisol, 5 α -tetrahydrocortisol, 5 β -tetrahydrocortisone, and 5 α -tetrahydrocortisone) according to methods previously reported (7, 8). In brief, the method consisted of enzymatic hydrolysis of urine sample, extraction, derivative formation, purification, GC/MS-SIM analysis, and quantification. The sum of 5 β -tetrahydrocortisol, 5 α -tetrahydrocortisol, 5 β -tetrahydrocortisone, and 5 α -tetrahydrocortisone was calculated as mg/g creatinine.

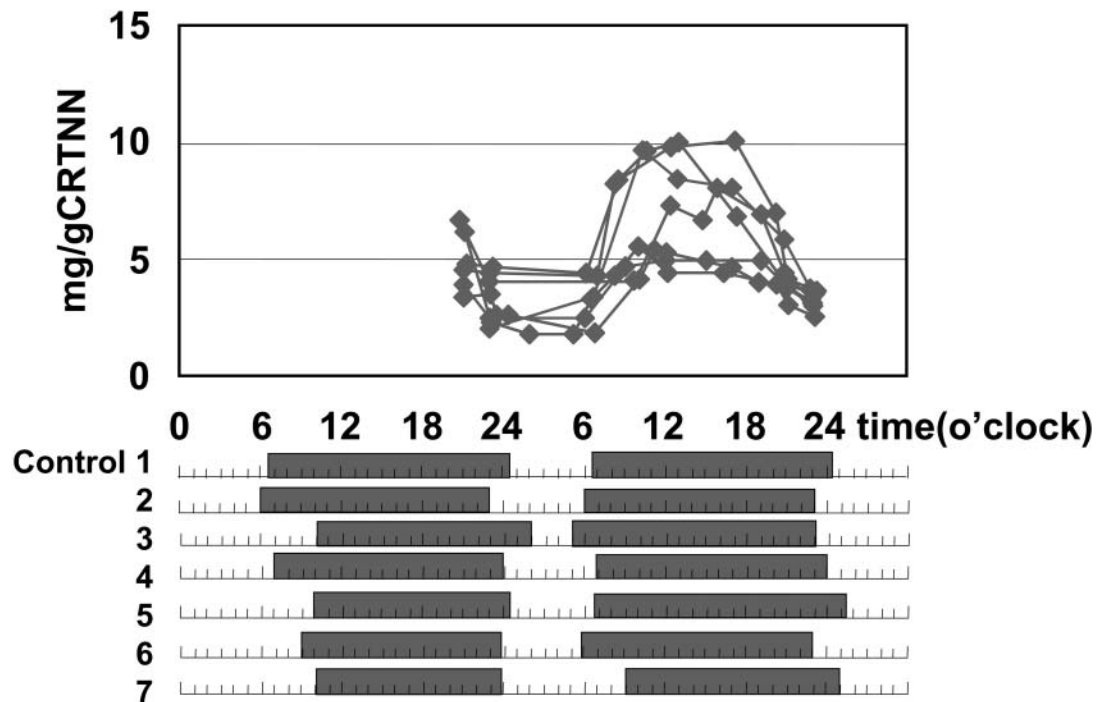


Fig. 1 The sum of cortisol metabolites in healthy young adults. The sum of 5β -tetrahydrocortisol, 5α -tetrahydrocortisol, 5β -tetrahydrocortisone and 5α -tetrahydrocortisone was calculated and plotted for each of the seven healthy young adults. Lower panel shows the sleep-wake patterns, which were recorded by the subjects. The gray scale indicates waking hours.

Results

Healthy young adults

All 7 healthy young adults showed circadian variation of the sum of cortisol metabolites. The levels of the sum were highest on waking, gradually declining throughout the day, and reaching nadir in the evening (Fig. 1). The tentative reference range of the sum of cortisol metabolites is shown in Fig. 2.

AN in childhood and adolescence after recovery of body weight by treatment (Fig. 3)

All 5 patients with AN in childhood and adolescence showed circadian variation of the sum of cortisol metabolites, being highest on waking, gradually declining throughout the day, and reaching nadir in the evening, the same as

in healthy young adults. In 4 out of 5 patients, the circadian variation was within the tentative reference range. One patient showed a somewhat low sum of cortisol metabolites during the daytime.

Discussion

We successfully analyzed the possible circadian variation of cortisol secretion in healthy young adults as well as patients with anorexia nervosa in childhood and adolescence after recovery of body weight by treatment using the sum of cortisol metabolites in urine using GC/MS in SIM. As far as we know, this is the first study on circadian variation of cortisol secretion as measured by GC/MS-SIM in any setting. Healthy young adults showed circadian variation of

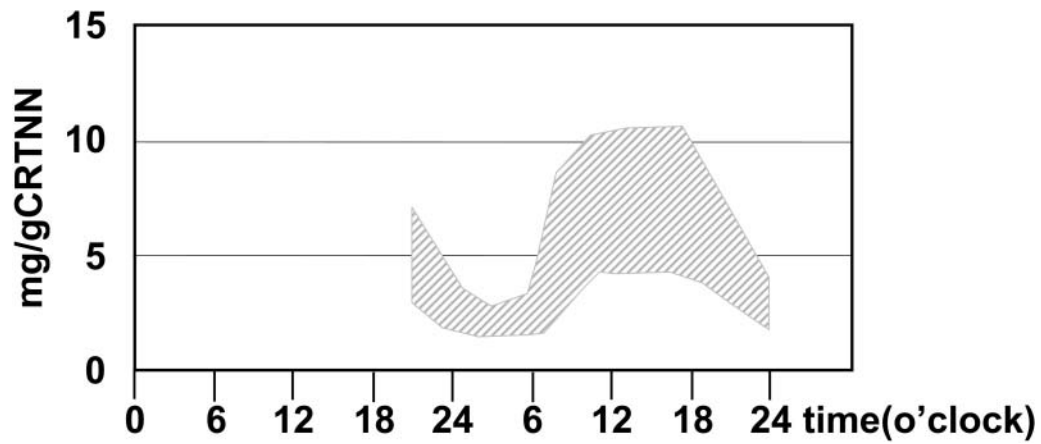


Fig. 2 The tentative reference range of the sum of cortisol metabolites. The maximum to the minimum of the sum was tentatively defined as the reference range and marked using oblique lines.

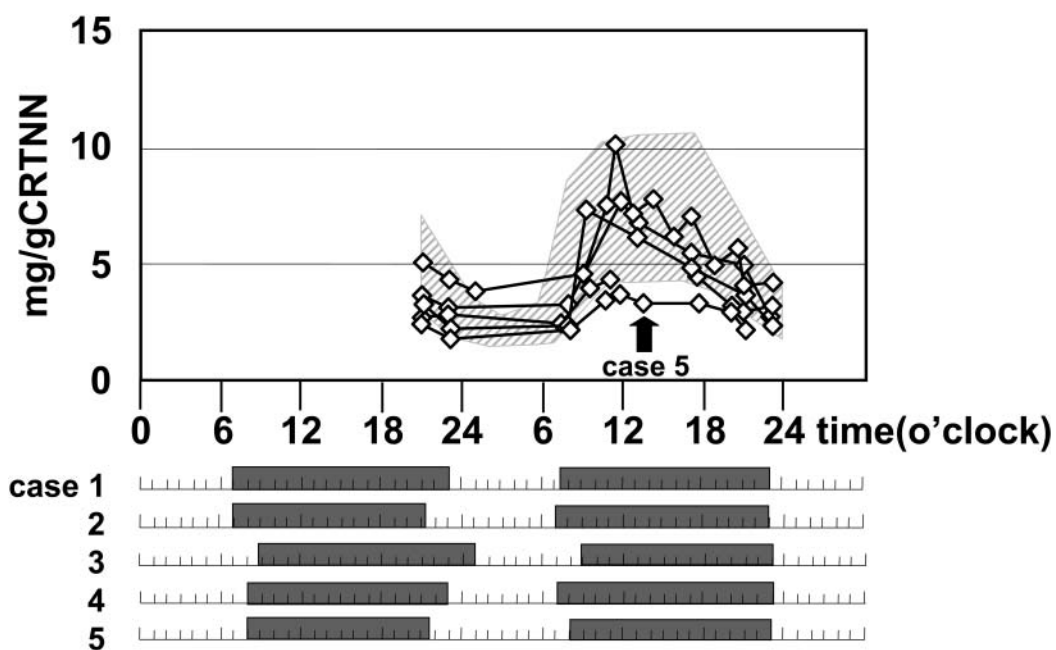


Fig. 3 The sum of cortisol metabolites in patients with AN in childhood and adolescence. The sum of 5β -tetrahydrocortisol, 5α -tetrahydrocortisol, 5β -tetrahydrocortisone and 5α -tetrahydrocortisone was calculated for the 5 patients with AN in childhood and adolescence. The data were plotted on the tentative reference range (Fig. 2). The arrow indicates Case 5. Lower panel shows the sleep-wake patterns, which were recorded by the subjects.

cortisol secretion, it being highest on waking, gradually declining throughout the day, and reaching nadir in the evening, which was consistent with a previous study showing circadian rhythm of plasma cortisol levels (14). The sum of cortisol metabolites in urine using GC/MS in SIM can be clinically applicable to subjects with any kind of disease considering its non-invasiveness and ease of collection compared to frequent blood sampling.

Our study indicates that patients with AN in childhood and adolescence after recovery of body weight by treatment showed the same circadian variation of cortisol secretion, as in healthy young adults. Little has been known about circadian variation of cortisol secretion in patients with AN in childhood and adolescence. Gold *et al.* reported that the circadian variation of plasma cortisol level in patients with AN in childhood and adolescence was conserved, while its level was often high (15). In terms of the circadian variation of cortisol secretion, our findings were consistent with their report, although our patients had been treated and their body weights had recovered. Further study is required to know if the circadian variation of cortisol secretion is conserved in patients with untreated AN in childhood and adolescence.

Our study had some limitations and perplexing points. First, the sum of cortisol metabolites in urine might not be the best parameter to analyze the circadian variation of cortisol secretion, considering the possible delayed metabolism of cortisol in AN, especially in untreated and underweight patients. Second, control subjects were young adults, whose ages differed from the patients with AN. Third, the numbers of controls and patients was not enough. Fourth, untreated and underweight patients with AN in childhood and adolescence were not included. Taken together, we are currently trying to increase the number of the control including teenagers and the number of patients including the untreated. Fifth, one patient with AN showed

somewhat low sum of cortisol metabolites in the daytime, which may be interpreted as “cortisol hyposecretion”. The reason for this result is unknown and this patient should be followed.

In conclusion, the sum of cortisol metabolites in urine by GC/MS in SIM was useful for analyzing the circadian variation of cortisol secretion. Although our data were preliminary, the circadian variation of cortisol secretion may be conserved in patients with AN in childhood and adolescence after recovery of body weight by treatment.

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