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Short Communication

Effect of Snack Eating on Sensitive Salivary Stress Markers Cortisol and Chromogranin A

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Abstract

Objectives: To investigate the effect of snack eating on salivary cortisol and chromogranin A (CgA).

Methods: From 14:00 to 18:00, starting two hours after consumption of a midday meal, saliva samples were collected every 30 minutes from 15 healthy males, 7 of whom (snack group) ate a snack immediately after the sampling at 15:00. Salivary cortisol and CgA levels were determined by ELISA. Samples were controlled according to salivary flow rates.

Results: For the snack group, after snack consumption, salivary cortisol increased to exceed significance ($p<0.05$) at 15:30 and rose even higher at 16:00. In the control group, there was no such change. There was no significant change in salivary CgA in either the snack group or the control groups during the sampling period.

Conclusions: These findings suggest that no food should be consumed for at least 90 mins before saliva sampling for cortisol determination and that salivary CgA is probably not affected by snack eating.

Key words: cortisol, chromogranin A (CgA), stress marker, human saliva, snack eating

Introduction

Evaluation of physiological stress markers in saliva, such as cortisol or CgA (chromogranin A), is a very useful method for objectively assessing stress. Furthermore, collection of saliva is a convenient sampling method because it is noninvasive and relatively non-stressful (1).

Saliva sampling to evaluate stress enables the investigation of people in free-living conditions in various circumstances. Under free-living conditions, however, the management of eating and drinking between meals is very difficult. Subjects are usually requested to refrain from eating and drinking before saliva sampling, but, as far as the authors know, there is no generally agreed time period for refraining from food consumption before saliva sampling.

In the present study, therefore, we tried to determine an appropriate abstention time before saliva sampling by investigating the effect of food consumption between meals on salivary cortisol and CgA.

CgA is an acidic glucoprotein that is known to localize in the secretory granules of a wide variety of endocrine and neuronal tissues (2–4). In particular, the level of salivary CgA provides a sensitive and reliable index for evaluating psychological stress (5). Moreover, levels of cortisol in saliva accurately reflect the free fraction of cortisol in plasma (1).

Materials and Methods

The subjects were 15 healthy male students at Osaka University. None were smokers or were on any medication. Before the experiment, mental health status was evaluated using GHQ-28 (6) and Zung-SDS (7, 8), while HPI (health practice index) (9, 10) scores were measured to assess lifestyle.

All subjects consumed a similar midday meal starting at 12:00. They were then randomly assigned to two groups: a snack eating group (snack group) and a control group. From 14:00 to 18:00, saliva samples were collected every 30 minutes using Salivette® (Sarstedt Co. Ltd., Nümbrecht). This device extracts saliva samples by centrifuging (at 3,000 rpm for 15 min) the cotton wads that subjects held in their mouths (for

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Table 1 Subject characteristics

Group	Number of subjects	Values (mean±SD)					
		Age (yrs)	Height (cm)	Body weight (kg)	GHQ-28	Zung-SDS	HPI
Snack group	8	23.8±7.9	173.7±7.3	71.1±7.8	5.4±4.4	38.4±9.3	5.1±1.4
Control group	7	24.0±5.1	174.4±5.5	70.4±8.5	4.3±3.5	38.4±7.1	5.6±1.5

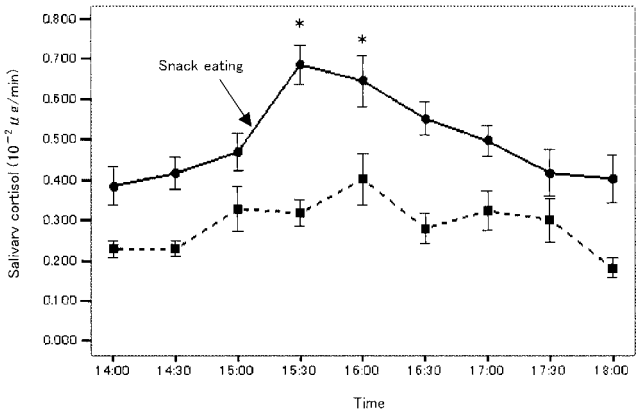

Fig. 1 Mean values (±SE) for salivary cortisol in snack group (●) and control group (■) during the sampling period. * Significantly different from 14:00, $p<0.05$ (repeated measures ANOVA and Dunett's test).

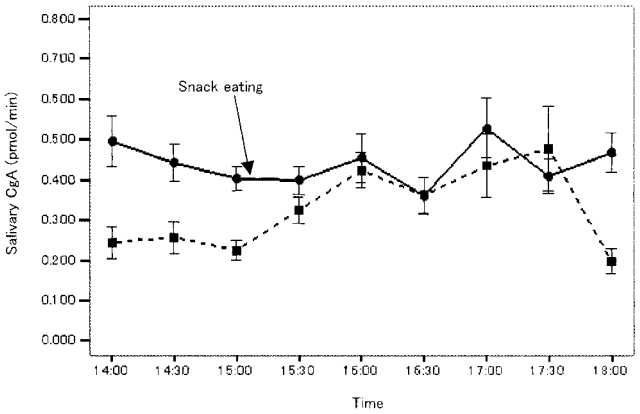

Fig. 2 Mean values (±SE) for salivary CgA (chromogranin A) in snack group (●) and control group (■) during the sampling period.

2 min). Immediately after the saliva sampling at 15:00, the snack group consumed two pieces of pound cake and a cup of tea (450 kcal). Throughout the experimental period, while they were present in our laboratory, all the subjects appeared to be in a relaxed condition. The samples were stored at −80°C until the assay.

The salivary cortisol and CgA levels were determined by ELISA using a previously described method (11, 12). To allow for increased salivation associated with snack eating, resultant values were adjusted according to salivary flow rates (ml/min). The salivary flow rates were calculated from the weight of saliva samples. Here, we defined the specific gravity of the saliva as 1 (13). The cortisol and CgA quantities per minute were calculated by multiplying salivary cortisol/CgA levels by salivary flow rates (14).

ANOVA with repeated measures was performed to detect inter-group and time-related differences. Dunett's test was used for multiple comparisons. Values were considered to be significantly different if $p<0.05$.

Results

Table 1 shows the basic characteristics of each group. There was no significant difference in age, physical characteristics, lifestyle, or mental health status between the two groups.

Snack eating affected the amount of salivary cortisol in the snack group: a significant increase in the amount cortisol present in saliva was detected in samples taken at 15:30, half an hour after snack eating ($p<0.05$). Even higher levels were detected from samples taken 16:00. Meanwhile, during the sampling period, in control group samples, no significant change in the amount of salivary cortisol was detected.

Fig. 2 shows the effect of snack eating on the amount of salivary CgA. During the sampling period, in both the snack and the control group, there was no significant change in the salivary CgA.

Discussion

In the snack group, there was a significant increase in salivary cortisol after snack eating (15:30). Detected levels were even higher at 16:00. Some previous studies of serum cortisol have found an abrupt rise of cortisol, co-occurring with the onset of food intake and reaching a peak within 1 hr. (15, 16). In addition, the same studies also found that food abstinence affects both the timing and the magnitude of the cortisol peak. In humans, therefore, food intake seems to correlate with the exogenous synchronization and amplification of cortisol secretion (15). In addition, the present findings suggest that the transport of the cortisol from serum to saliva is immediate.

CgA is known to be co-released with catecholamines during exocytosis (2, 3). A previous study found significantly increased catecholamine levels after food consumption (16). In the present study, however, we found no significant change in salivary CgA related to food consumption; a previous study has suggested that there is not always a linear relationship between plasma catecholamine and salivary CgA levels (5). Further investigation of the correlationship of CgA and catecholamines is required to confirm our findings.

The salivary cortisol and CgA at baseline (14:00) were slightly higher in the snack group. It has been suggested that, in addition to mental health status, cortisol levels may be affected by various lifestyle factors (17). As described above, both the groups that we studied were demographically similar. In addition, all the subjects were healthy nonsmokers and none were on any medication. The differences between baseline cortisol

and CgA, therefore, may result from the natural individual variation.

The present findings suggest two things. First, that an abstention period of at least 90 mins should be enforced before saliva sampling for cortisol measurement. Second, that salivary CgA is not affected by snack eating. To conclusively establish these assumptions, further studies are required.

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