



# Effects of woodland walking on salivary stress markers cortisol and chromogranin A

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## KEYWORDS

Chromogranin A (CgA);  
Cortisol;  
Forest;  
Human saliva;  
Walking

## Summary

**Objective:** We investigated the effect of walking through woodland on salivary endocrinological stress markers, cortisol and chromogranin A (CgA).

**Design:** From 20 healthy males, saliva samples were collected immediately before and after the walk, and 20 min and 40 min after that. In addition, using visual analog scales, subjective perceptions of stress and of feeling tired and uplifted were evaluated. On another day, as a control, they sat around in their office and samples were collected at the same times. Salivary cortisol and CgA levels were determined by enzyme-linked immunosorbent assay (ELISA).

**Results:** Samples taken after the walk showed a significant increase in the levels of CgA. Meanwhile, there was no significant change in the levels of cortisol. In control samples, there was a statistically significant decrease in the levels of both CgA and cortisol. Feelings uplifted and tired increased and subjective perception of stress decreased significantly after the walk. During the control period, there were no such changes.

**Conclusion:** These findings suggest that, while walking in woodland becomes a physical stressor, such activity may also bring about feeling uplifted.

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## Introduction

In Japan, the effects of forest walking are once again being recognized as complementary and alternative medicine. It is believed that as people walk, they breathe in beneficial volatile compounds emitted by the forest. This activity

is characterized as a kind of climatherapy or aromatherapy. A previous study, whose authors glossed the activity in 'forest-air bathing,' found lower blood glucose levels in diabetic patients who had walked in a forest.<sup>1</sup> Furthermore, phytoncides, volatile substances released from trees, have been found to induce human natural killer cell activity in vitro<sup>2</sup> and to reduce blood pressure in stroke-prone spontaneously hypertensive rats.<sup>3</sup> Moreover, previous studies revealed that individuals staying in a forest area had lower levels of salivary cortisol than individuals staying in a city area.<sup>4–6</sup> Apart from these reports on male university students, to our knowledge, no research into the effects of

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walking in a wooded ambience on endocrinological stress markers has been conducted. We think, however, that for the elderly as well as the young, the ambience would be useful to health promotion. Consequently, in the present study, we examined how a walk through woodland affected the salivary endocrinological stress markers cortisol and chromogranin A (CgA) in elderly probands.

Endocrinological stress markers are useful for assessing the activity of a specific stress system. Their presence in saliva samples can be assayed, and the collection of saliva is a noninvasive, relatively nonstressful, and, consequently, highly convenient sampling method.<sup>7</sup> Playing a central role in physiological adaptation to various stressors, cortisol is an adrenocortical hormone that is secreted via the hypothalamus–pituitary–adrenal (HPA) axis. The level of cortisol in the saliva accurately reflects the level of active free cortisol in the blood.<sup>7,8</sup> Previous studies have reported that aging can lower morning cortisol levels, which is likely linked to decreased hippocampal functioning.<sup>9–11</sup> Even so, this does not influence the assay of cortisol.<sup>12</sup>

Meanwhile, CgA is an acidic glucoprotein that is released along with catecholamines from the adrenal medulla and the sympathetic nerve endings.<sup>13–15</sup> A recent study has reported that CgA is produced by human submandibular glands and secreted into saliva.<sup>16</sup> Salivary CgA has gained attention as a novel stress marker. Previous studies have found that salivary CgA levels are statistically significantly increased by mental stress caused by activities such as public speaking, word processing tasks, or driving a car on an expressway.<sup>17,18</sup> Moreover, very recent studies have found significantly increased salivary CgA levels after high-intensity exercise such as incremental maximal swimming test or treadmill stress test.<sup>19,20</sup> In addition, in healthy subjects, salivary CgA levels seem to be unaffected by age.<sup>21</sup>

## Materials and methods

After receiving the approval of the institute's review board, we enrolled randomly 20 healthy male volunteers (64–74 years) belonging to a nonprofit organization, the Senior Nature College. Prior to the study, written informed consent was received from each participant. They had to take a 1000m walk at their own pace from the start of a mountain path to an observation platform (elevation gain: 260m) within the forest on Ikoma Mountain at the eastern edge of Osaka Prefecture, Japan. As Fig. 1 shows, saliva samples were collected immediately before (10:15) and after the walk (11:00), and 20 min (11:20) and 40 min after walking finished (11:40). Before the first sampling (10:15), as a warming-up exercise, they did stretches of their own accord for 2–3 min. On another day, they sat around in their

office and saliva samples were collected at the same times. Thus each subject acts as his own control. Systolic and diastolic blood pressure and heart rate were measured using an automatic digital sphygmomanometer HEM-630 (OMRON Corp., Kyoto, Japan). In addition, using ten-division visual analog scales, subjective perceptions of stress and of feeling tired and uplifted were evaluated.

Saliva samples were collected using the Salivette system (Sarstedt Co., Ltd., Nümbrecht, Germany). In this system, subjects hold cotton wads in their mouths for 2 min and the saliva in the wads is later extracted by centrifuging (at  $3000 \times g$  for 15 min). Subjects were requested to refrain from eating and drinking during the 2 h before sampling began at 10:15 until after the final sample was taken at 11:40.<sup>22</sup> The samples were stored at  $-80^{\circ}\text{C}$  until the assay. Using enzyme-linked immunosorbent assay (ELISA), following a previously described method,<sup>23,24</sup> we evaluated the levels of salivary cortisol and CgA.

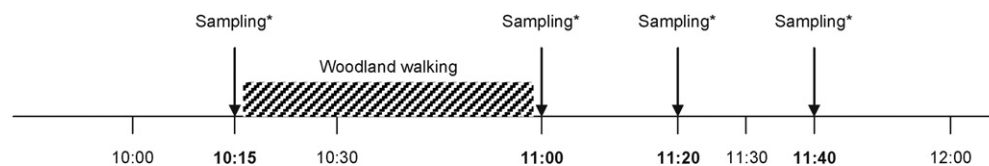
All results were displayed as mean values  $\pm$  standard deviation. Student's paired *t*-test was performed to detect interprotocol differences, and ANOVA with repeated measures to detect time-related differences. Dunnett's test was used for multiple comparisons. Statistically significant differences between values was indicated when  $p < 0.05$ .

## Results

The mean age, body weight, height, and body mass index (BMI) of the subjects were  $67.6 \pm 2.8$  years,  $64.3 \pm 6.3$  kg,  $165.8 \pm 5.9$  cm, and  $23.4 \pm 1.4$  kg/m<sup>2</sup>, respectively. Table 1 shows the baseline (before the walk) levels of each parameter. Heart rate was significantly higher in the walking protocol than in the control protocol ( $p < 0.01$ ).

In samples taken immediately after the walk, we detected significantly higher levels of salivary CgA than in those taken before the walk ( $p < 0.05$ ) (Fig. 2). There was no such change in the levels of salivary cortisol. In the control protocol, levels of salivary CgA were significantly lower in samples taken 40 min after the walk than in those taken before the walk ( $p < 0.05$ ). Meanwhile, levels of salivary cortisol were significantly lower in samples taken 20 min ( $p < 0.05$ ) and 40 min after the walk ( $p < 0.05$ ) than in those taken before the walk. At 40 min after the walk, salivary CgA levels were significantly lower in the control protocol than in the walking protocol ( $p < 0.05$ ). In addition, at 20 min after the walk, salivary cortisol levels were significantly lower in the control protocol than in the walking protocol ( $p < 0.05$ ).

Systolic ( $p < 0.05$ ) and diastolic blood pressure ( $p < 0.05$ ) decreased significantly immediately after the walk compared to before the walk (Fig. 3). At



**Figure 1** Study protocol. \*Sampling includes: saliva sampling, measurement of systolic and diastolic blood pressure and heart rate, and using 10-division visual analog scales to evaluate subjective stress, and feeling uplifted and tired.

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