Acta Med. Nagasaki 62: 49－54

Exposure to Essential Oil Odors Increases Salivary Testosterone Concentration in Perimenopausal Women.

Wataru TARUMI[1], Chizu KUMAGAI[2], Kazuyuki SHINOHARA[1]

[1] Nagasaki University Graduate School of Biomedical Sciences, Department of Neurobiology & Behavior, Nagasaki University 1-12-4 Sakamoto-cho, Nagasaki 852-8523, Japan, TEL: +81-95-819-7033; FAX: +81-95-819-7036; E-mail: tarumi@nagasaki-u.ac.jp

[2] Aroma Environment Association of Japan,1-5-20-6F Yaesu, Chuo-ku Tokyo103-0028, Japan TEL+81-3-3548-3401; FAX: +81-3-3548-3402; E-mail: reseach@aromakankyo.or.jp

The current studies have shown that some menopausal symptoms are due to a reduction in testosterone levels. However, testosterone replacement therapy is not the best option for menopausal women because possible occurrence of side effects due to testosterone replacement therapy cannot be ignored. Therefore, in order to explore a possibility that olfactory exposure to essential oils increase testosterone release in menopausal women, we measured salivary testosterone concentration before and after olfactory stimulation by the essential oils.

This experiment was conducted in a laboratory dedicated to odor exposure experiments and in a ventilate laboratory at Nagasaki University. In order to minimize the influence of visual stimuli on the subjects, the room lighting was turned off and light from the outside was shielded by a curtain.

Perimenopausal women (n = 15 per essential oil) were exposed first to propylene glycol or dipropylene glycol as a control and then to 1 of 10 essential oils (geranium, rose otto, orange sweet, lavender, neroli, frankincense, jasmine absolute, ylang ylang, roman chamomile, and clary sage) dissolved in propylene glycol or dipropylene at 2 l/min for 20 minutes on each experimental day. We measured the testosterone concentrations in saliva before and after each odor exposure in these perimenopausal women.

The results of this study demonstrate that olfactory stimulation by jasmine absolute, roman chamomile, and clary sage essential oils increased salivary testosterone. The clinical significance of this should be examined an empirical study in the future.

ACTA MEDICA NAGASAKIENSIA 62: 49－54, 2019

Key words: Perimenopause, Essential Oil, Testosterone, Jasmine absolute, Roman chamomile, Clary sage

Introduction

Climacteric is the transitional period from the reproductive to the post reproductive phase, lasting up to 10 years before and after menopause.[1] The mean age at menopause is 50.5 years, with the climacteric period occurring between 40 and around 60 years old.[2] Climacteric symptoms often include mental symptoms characterized by reduced sexual function, increased anxiety, depression irritability and impairments in memory, and physical symptoms characterized by hot flashes, sensation of heat in the face, headache, general malaise, vaginal atrophy, osteoporosis, and heart disease.[3,4] Although the occurrence of these menopausal symptoms can be mostly attributed to a reduction in estrogen levels, some may be due to a decrease in testosterone.[5]

A woman with a physiologically normal menstrual cycle is thought to produce approximately 300 μg of testosterone a day, half of which is produced by the ovary and the other half by the adrenal glands.[6,7] At menopause, the testosterone concentration in blood drops to 50% of the concentration

Address correspondence: Kazuyuki Shinohara, Nagasaki University Graduate School of Biomedical Sciences, Department of Neurobiology & Behavior, Nagasaki University 1-12-4 Sakamoto-cho, Nagasaki 852-8523, Japan, TEL: +81-95-819-7033; FAX: +81-95-819-7036; E-mail: kazuyuki@nagasaki-u.ac.jp

Received July 30, 2018; Accepted October 22, 2018

prior to menopause.[8,9] This reduction in testosterone during the menopausal transition can reduce memory function, sexual desire, and feelings of happiness, but it has not yet been clarified how testosterone decreases cause menopausal symptoms.[10-14]

Many lines of evidence suggest the effectiveness of testosterone replacement therapy in improving the sexual desire in post-menopausal women. However, the therapy may be accompanied by side effects such as acne and hirsutism, as well as heart disease and diabetes mellitus as a result of hyperandrogenism.[15-17] Furthermore, the safety of long-term testosterone replacement therapy has not been reported. Therefore, testosterone replacement therapy is not clinically recommended to treat climacteric disorders at least in Japan, so that there has been a need for an alternative medicine, which can facilitate the patients to intrinsically increase testosterone levels.[18]

Aromatherapy could be a candidate of alternative medicine for the treatment of climacteric disorder since exposure to some essential oils ease impairments in cognitive functions, including memory, sexual function and feeling of happiness.[19-22] It has been shown that aromatherapy may alleviate the menopausal symptoms scored by Kupperman index.[23,24] Furthermore, exposure to essential oils has been proved to increase salivary estrogen concentration in perimenopausal women.[23,24] Therefore, the essential oils may increase estrogen levels to ease some of menopausal symptoms. It is, however, possible that these essential oils may not relieve menopausal symptoms associated with reduction in testosterone.

The aim of the present study is to determine which of 10 essential oils that may alleviate menopausal symptoms increase testosterone concentration in menopausal women. In this study, we compared salivary testosterone concentrations before and after olfactory exposure of essential oil to the perimenopausal women.

Materials and Methods

Participants

We measured the concentration of testosterone in saliva according to the method as shown in previous studies.[24] Fifteen subjects (average age 44.2 ± 2.7 years) were recruited to verify the effect of each essential oil on salivary levels of testosterone (See, Table1). One subject could not participate in the exposure experiment to jasmine absolute. All subjects had a menstrual cycle (21 to 37 days). There were no smokers among the subjects. All subjects provided informed consent prior to their participation in this study. This research was approved by the ethics committee of Nagasaki University and executed according to the Declaration of Helsinki. IRB research approval number is 13121970.

Table 1. Age and number of participants, who participated in the testing of each essential oil.

Essential oil	N	Age (yrs; avg ± sd)		
Jasmine absolute	14	44.8	±	2.8
Roman Chamomile	15	43.9	±	2.4
Clary sage	15	43.8	±	2.5
Geranium	15	44.1	±	2.8
Rose otto	15	44.3	±	2.9
Orange sweet	15	43.9	±	2.8
Lavender	15	44.4	±	2.6
Neroli	15	44.8	±	2.8
Frankincense	15	44.2	±	2.9
Ylang Ylang	15	44.2	±	2.5

Source: Shinohara K et al, Neuro Endocrinol Lett. 2017

Chemicals

In this study, we used 10 essential oils: geranium, rose otto, orange sweet, lavender, neroli, frankincense, jasmine absolute, ylang ylang, roman chamomile, and clary sage. Ylang ylang essential oil was purchased from Tree of life Co., Ltd. (Tokyo, Japan), and the other essential oils were purchased from Neal's Yard Remedies (London, UK). We exposed essential oil diluted by propylene glycol or dipropylene glycol, and propylene glycol or dipropylene glycol only as a control, as previously reported (see, Table 2).[24]. Information on dilution liquid and concentration of each essential oil is shown in Table 2. Further, only an orange sweet wasn't diluted, and dipropylene glycol was used as it's control.

Table 2. The solvent and concentration of each essential oil used in the present study.

Essential oil	Conc. (%)	Solvent
Jasmine absolute	12	dipropylene glycol
Roman chamomile	0.75	dipropylene glycol
Clary sage	12	dipropylene glycol
Geranium	3	propylene glycol
Rose otto	12	propylene glycol
Orange sweet	100	—
Lavender	30	dipropylene glycol
Neroli	7.5	dipropylene glycol
Frankincense	60	dipropylene glycol
Ylang ylang	1.5	dipropylene glycol

Apparatus for Odorant Exposure

The apparatus for odorant exposure experiments consisted of an air pump, flow meter, glass bottle, silicone tube, and glass funnel. The glass bottle was filled with 15 ml of diluted essential oil or solvent. The flow rate was set at 2.0 L/min using a flowmeter. The air was pumped into the glass bottle and the air flow from the bottle was set close to the participants nostrils allowing exposure by a silicone tube. The glass funnel helps to reduce the discomfort caused by the air current blowing on the participants.

Procedure

An experiment was conducted between 5 to 10 days after the start of menstruation. When the subjects arrived at the laboratory, they were kept in a relaxed state for 15 minutes before the start of experiments in order to stabilize their psychological state. The experimental procedure of this study is the same as that published in the previous study,[24] where subjects first smelled the control solvent (20 minutes) and then smelled 1 of 10 essential oils (20 minutes). This procedure is not repeated in a single day, meaning that subjects smelled only one essential oil per day. In addition, the order of the essential oil exposure was randomized. Saliva was collected before and after odorant exposure. In order to minimize the influence of visual stimuli on the results, the room lighting was turned off and light from the outside was shielded by a curtain. The saliva samples were kept at -80 degrees Celsius until used for analysis.

Testosterone Assay

Saliva samples were thawed completely and centrifuged at 1,500 × g for 15 min at room temperature. The testosterone concentration in the saliva was measured using a competitive ELISA kit (High Sensitivity SALIVARY TESTOSTERONE ENZYME IMMUNOASSAY KIT; Salimetrics LLC, Carlsbad, California, USA) according to the protocols recommended by the manufacturer.

Analyzing

In order to control diurnal variation of salivary testosterone concentration in the experimental day, the variation rate of testosterone concentration was computed by dividing the testosterone concentration in the post-exposure by that in the pre-exposure. 25 To clarify which essential oil affected the level of salivary testosterone, the variation rate of salivary testosterone concentration was analyzed by paired t-test for comparison of the effect of each essential oil versus the control. In all analyses, $P < 0.05$ was considered to indicate statistical significance.

Results

The analyses revealed that significant increase in the variation rate of salivary testosterone concentration in essential oil exposure was observed for jasmine absolute, roman chamomile and clary sage (Table 3), compared with control odor exposure. No significant difference was observed for other essential oil exposure (Table 3).

Table 3. Averages and standard deviations, and 95% confidence interval of variation rate of salivary testosterone concentration in each condition.

	Control	95% C.I.	Essential oils	95% C.I.	P-Value
Jasmine absolute	1.00 ± 0.22	[0.87, 1.12]	1.15 ± 0.13	[1.07, 1.22]	0.01*
Roman chamomile	1.03 ± 0.20	[0.92, 1.14]	1.26 ± 0.28	[1.10, 1.41]	0.02*
Clary sage	1.00 ± 0.09	[0.95, 1.05]	1.11 ± 0.14	[1.03, 1.19]	0.02*
Geranium	1.06 ± 0.15	[0.98, 1.14]	1.10 ± 0.15	[1.01, 1.18]	0.43
Rose otto	1.02 ± 0.20	[0.91, 1.13]	1.07 ± 0.16	[0.99, 1.16]	0.37
Orange sweet	1.06 ± 0.21	[0.94, 1.18]	1.05 ± 0.15	[0.97, 1.13]	0.9
Lavender	1.01 ± 0.26	[0.87, 1.15]	1.04 ± 0.22	[0.92, 1.16]	0.73
Neroli	1.07 ± 0.25	[0.93, 1.21]	1.11 ± 0.18	[1.00, 1.21]	0.58
Frankincense	0.93 ± 0.19	[0.83, 1.04]	1.10 ± 0.30	[0.94, 1.27]	0.07
Ylang Ylang	0.97 ± 0.23	[0.85, 1.10]	1.05 ± 0.27	[0.91, 1.20]	0.22

C.I.: Confidence Interval.
** P<0.05 vs. Control.*

Discussion

The current study revealed that olfactory exposure of jasmine absolute, roman chamomile, and clary sage essential oils increased salivary testosterone concentration in perimenopausal women. Together with our previous report that essential oils increased estrogen, current study suggests that olfactory stimulation of some essential oils increase gonadal steroid hormones in perimenopausal women whose ovarian function may deteriorate.

In the current study, the increases in salivary testosterone concentrations were found within 20 minutes after the start of olfactory stimulation by essential oils. It has also been shown that human odors changed testosterone level as quickly as in the current study. Hence, the salivary concentrations of testosterone were increased in men 15 minutes after smelling the scent of a woman[27] and was decreased 15 minutes after the start of smelling the scent of women tears[28]. In a similar experimental protocol to current study, we have reported that essential oils increased estrogen level 20 min after the start of exposure.[24] A significant increase in estrogen concentrations was also observed in women who are exposed to saffron oil for 20 minutes[26]. Based on these studies, 20 min exposure to acute olfactory stimulation may be enough to release gonadal steroid hormones in saliva, suggesting olfactory stimulation could be quick remedy for the menopausal symptoms.

It has been reported that some menopausal symptoms, such as the decline in sexual function, feelings of happiness and memory may be related to a decrease in the amount of testosterone secretion as a result of ovarian aging[29-32]. The current study, therefore, suggest that these essential oils could alleviate menopausal symptoms related with testosterone reduction. This research, however, merely evaluated acute effects of essential oil on endocrine function. In order to promote the clinical use of the three essential oils for menopausal symptoms, it is necessary to evaluate effectiveness and safety of chronic effect of the essential oils.

In the current study, we could not clarify the reason why only 3 of 10 essential oils increased in testosterone. It is, however, possible that the 3 oils share a specific odorant component, which increases the salivary concentration of testosterone. Furthermore, the testosterone increase may be caused by a combination of a several odorant components contained in the essential oil.[33-36] The former possibility is less likely because there is no single odorant component shared among jasmine absolute, roman chamomile, and clary sage essential oils as shown in our previous reports. [24]

We were unable to clarify the clinical implications of the increase in testosterone in the experimental model of this study. However, female sexual function is reported to be related to testosterone concentrations.[38,39] A reduction in testosterone concentrations is associated with decreased sexual intercourse and libido in women.[40] It is also known that anti-androgens have a detrimental effect on their sexual function and that testosterone levels were decreased in women with decline of sexual function.[41] Furthermore, many studies have reported that sexual function was improved in women who received testosterone.[42-45]

Testosterone may exert an effect not only on sexual function but also on memory and mental function. It was also reported that, when testosterone was administered in combination with estrogen, compared to administering estrogen alone, a more refreshing and energetic feeling was obtained in women with surgically-induced menopause.[46,47] For example, in those with surgically-induced menopause, the Psychological General Well Being Index improved with percutaneous testosterone replacement therapy.[45] These studies have demonstrated the beneficial effects of testosterone in perimenopausal women.

The average and standard deviation of salivary testosterone concentration before odor exposures was 71.0 pg/ml and 28.24, respectively. These values were similar to those in perimenopausal women living in Britain.[48] In that report, adolescent male salivary testosterone levels were twice as high as those in adolescent females, which is consistent with our previous findings.[49] These reports suggest that the salivary testosterone concentrations in the present study are physiologically reasonable.

There are some limitations in the present study since the present study is a feasibility study for screening essential oils that have the potential to increase testosterone secretion. First of all, the essential oils used in the present study releases an inherent flavor, so it is impossible to do a blind study. Therefore, it can't be denied possibility that personal preference for odor affected the results. Second, in order to prevent residues of the essential oil from affecting the experimental results, the exposure to the essential oil was always preceded by exposure to the control odor. Therefore, we cannot deny the possibility that the order of exposure affected the results. Furthermore, we did not collect basic subject information (i.e. BMI, baseline testosterone levels, measurement of the "relaxed state" prior to exposure to an odorant, climacteric symptoms) in the present study.

A significant increase in testosterone levels was detected in groups exposed to jasmine, chamomile, and clary sage in the present study. Furthermore, while frankincense exposure did not increase testosterone significantly, the group exposed

to frankincense showed a significant trend (frankincense: 1.10 ± 0.30 vs. Control: 0.93 ± 0.19, P=0.07). Given the small number of subjects participating in this study, further experiments should be conducted before concluding whether or not frankincense increases salivary testosterone levels.

In summary, the results of this study demonstrate that olfactory stimulation by jasmine absolute, roman chamomile, and clary sage essential oils increased salivary testosterone. The clinical significance of this should be examined an empirical study in the future.

Acknowledgement

This work was supported by the Aroma Environment Association of Japan's Research Grant. We would like to thank Yoko Komori and Yuka Nakazawa for their technical assistance.

Disclosure Statement.

No competing financial interests exist.

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