Original Article

Female Pheromone and Physical Exercise Improve Endocrine Status in Elderly Japanese Men

Akira Tamagawa ¹⁾, Ludmila A. Gerlinskaya ²⁾, Ryoichi Nagatomi ¹⁾, Mikhail P. Moshkin ²⁾
¹⁾ Department Medicine and Science in Sports and Exercise, Graduate School of Medicine, Tohoku University, Sendai (Japan)

²⁾ Institute of Systematics and Ecology of Animals, Siberian Branch of RAS, Novosibirsk (Russia)

Abstract

BACKGROUNDS: Declines in muscle mass with age are closely related to changes in the balance between anabolic and catabolic hormones, probably due to the progressive decrease in circulating testosterone. There are some factors to affect the anabolic to catabolic hormone ratio. For example, anabolic to catabolic hormone ratio increases transitorily just after exercise. In addition, an increase in salivary testosterone in men exposed to female pheromones has been reported. Although exercise tolerability and olfactory perception decline with age, we can not exclude the possibility of the adaptation for exercise training and of the sensitivity for pheromone exposure in elderly men.

OBJECTIVE: To examine whether exposure to female pheromones and physical exercise can modulate anabolic and catabolic endocrine status in elderly Japanese men.

DESIGN: A random cohort, double-blind, placebo-controlled trial.

SETTING: Investigators tested subjects at community center in Tomiya Town, Miyagi, Japan.

PARTICIPANTS: Twelve 52 to 74 y-old Japanese men participating in a 5-week exercise class.

MEASUREMENTS: From the second to fifth exercise class periods a commercially available pheromone (Osmopherine) was applied to the upper lip of 6 men before exercise and continued as volatile molecules were released from necklace worn continuously. Another 6 men received the pheromone carrier gel on their upper lip and a necklace with only the vehicle. Saliva samples were collected before and after each exercise class to measure testosterone and cortisol concentrations. The daily physical activity level for each subject was assessed by an accelerometer.

RESULTS: Pheromone exposure and exercise classes led to a significant increase in the pre-exercise testosterone to the cortisol ratio ($F_{1,39} = 4.8$, $P=0.035$ $F_{4,39} = 12.0$, $P<0.001$, respectively). However, pheromone exposure did not affect the activity levels of participants.

CONCLUSION: Female pheromone exposure in combination with exercise classes modulated endocrine effects in older men.

KEY WORDS: aging, testosterone, cortisol ratio, exercise, female pheromone

Introduction

Declines in muscle mass with age are closely related to changes in the balance between anabolic (e.g., androgens, GH, IGF-I) and catabolic (cortisol) hormones, probably due to the progressive decrease in circulating testosterone. ¹⁻⁴⁾ Sarcopenia related to age results in a decrease of muscle strength, bone density, glucose tolerance and increased fatigability. ⁵⁻⁷⁾ Low levels of plasma testosterone also negatively influence mental performance and psychological status of older men. ^{8,9)} Testosterone supplements can correct physical and mental disability, but hormonal therapy has unfavorable effects, including gonad dysfunction, elevated hematocrit, increased prostate size. ¹⁰⁻¹²⁾

The side effects of testosterone supplements stimulated studies directed to working up drug-free approaches to improve the ratio of anabolic to catabolic hormones. One is physical exercise. ¹³⁾ Comparative studies of physically active and sedentary people demonstrated that lifestyle often, but not always, correlates with endocrine status in older man. ^{7,14)} Anyway special training courses

designed to improve the physical condition of elderly people had contradictory endocrine effects. Although anabolic to catabolic hormone ratio increased transitorily just after exercise, ¹⁵⁾ no long-term course was found effective for improving endocrine status. For example, 16 weeks of strength training lead to an increase in maximal strength and muscle power output of the arm and leg muscles in older men, but there were no changes in serum testosterone. ¹⁶⁾

Many experimental studies on mammals have found unequivocal evidence that smelling female sex pheromones can result in activation of the male hypothalamic pituitary gonad system. ¹⁷⁻¹⁹⁾ Most of these studies were performed for rodents which were delicate sense of smell, considered “macro-osmatics”. The human olfactory degenerates in comparison with the rodents, considered “micro-osmatics”. Although humans are referred to as “micro-osmatics”, some authors believe that airborne chemical signals play an important role in non-verbal communication between people. ²⁰⁻²²⁾ Evidence of modulations by pheromone exposure of the human endocrine system, autonomous nervous system, mood

and other behavioral traits have been summarized in several review papers published during the last 5 years.^{23,24)} In particular, Grammer and Jutte²⁵⁾ reported an increase in salivary testosterone in men exposed to copulins, which are described as female pheromones.

Relatively young men and women were the subjects in most of the previous studies. Although olfactory perception declines with age, we can not exclude the possible somatic and psychological effects of sex pheromones on the elderly. Recently, Friebely and Rako²⁶⁾ showed changes in the socio-sexual behavior in postmenopausal woman (about 57 years old), who habitually wore a fragrance “spiked” with a female pheromone manufactured by the Athena Institute for Women’s Wellness Research, Chester Springs, PA (code - 25 of pheromone formula). 68.2% of the women, who received pheromone, reported increased incidences of petting, kissing, and affection. This was significantly higher than the only 40.9% increase reported in the controls who received the placebo. These data indicate that the contemporary partners of these women who wore the fragrance were susceptible to female pheromones.

Based on the studies of Grammer and Jutte²⁵⁾ and Friebely and Rako²⁶⁾, we hypothesized an increase in the anabolic to catabolic hormone ratio in elderly men exposed to female pheromones. Because an organized male group provides suitable conditions for a controlled pheromone experiment, we studied the effects of pheromones on the hormones of elderly men who participates weekly exercise classes on Tuesdays. Salivary testosterone and cortisol were measured to calculate the anabolic to catabolic hormone ratio. The concentration of these hormones in saliva is strongly correlated with the variations of bioavailable free testosterone and free cortisol in blood plasma.^{27,28)}

Methods

Subjects

Twelve older males taking a standard physical training course volunteered with informed consent (reviewed by the Ethics Committee of Tohoku University: No2006-282) to participate in our study. The participants ranged from 52 to 74 years old (mean $\pm$ SD 64 $\pm$ 5 y). During the 5 weeks of our study, subjects had a 90 min exercise class on Tuesdays from 1900 to 2030 (local time). We used the first week to collect baseline data. From the second to the fifth class, 6 subjects selected at random were exposed to the pheromone. The other 6 were exposed to the placebo. Just before and after the classes, we collected within 15 min saliva samples to assess the pre- and post-exercise concentrations of testosterone and cortisol. Following a double-blind methodology, we did not inform the participants what type of exposure (pheromone or placebo) they received. Technical assistants who assessed the hormone concentrations were also blinded to the sample origin. In the 4th week of training, 3 participants missed the exercise class due to their having influenza. Those participants (2 from the pheromone group and 1 from the placebo group) were excluded from the analysis.

Pheromone

We used a commercially available pheromone preparation produced by Vevy Europe (Genoa, Italy) sold under the name Osmopherine. According to information from the company, Osmopherine belongs to the class of female sex pheromones (copulin). We used 0.02% Osmopherine diluted in alcohol (96%) for

the necklace vials. And one part of the Osmopherine solution into 9 parts of carrier gel (Aroma and Life Co., Japan) was prepared for applications to the upper lip. Osmopherine diluted in the carrier gel was applied to the upper lip of the pheromone-exposed men, just before class from week 2 through 5 of the exercise classes. The upper lip of men in the control group was greased by pure carrier gel, according to the same. After the classes, men of both groups were each given a necklace capsule with pure alcohol (control group) or Osmopherine diluted in alcohol. They wore the capsules at all times until the end of the experiment. We also provided each man with a vial containing alcohol or diluted pheromone. The participants were instructed to add 5 to 6 drops of fluid from the vial to the necklace capsule each morning.

Activity

The exercise classes, under the guidance of an experienced instructor, consisted of 20 min of stretching, a 10-min brisk walk, 10 min of strength training, 40 min of badminton and a 10-min cool down. Throughout the experiment, each subject wore an accelerometer (Knez Lifecorder EX activity monitor; Suzuken Co., Nagoya Japan) which measured the intensity and time spent on physical activity throughout daily life. We evaluated the activity of daily life, energy expenditure and step counts.

Salivary testosterone and cortisol

Subjects were asked to spit about 1.5 ml of saliva into a plastic tube after oral rinsing with distilled water. Following the recommendations of Granger, et al.²⁹⁾, we visually inspected the saliva samples for discoloration by blood. Three samples showing blood were rejected. Fortunately, these were samples from subjects who were excluded from analysis (see *Subjects*). The samples were centrifuged at 3000 rpm for 15 min. About 1 ml of each sample was placed into an Eppendorf tube and stored at -20°C. The samples were tested using an immuno-enzyme method with commercial kits (SteroidIFA-cortisol-01 and SteroidIFA-testosterone-1, ALKOR Bio, Saint-Petersburg, Russia) with enough sensitivity to detect testosterone and cortisol in 100 μ l saliva samples without previous any necessary extraction step. The measurements were made according to the kit instructions. Saliva free of steroids was used as a vehicle for the standard solutions of pure cortisol (Sigma, USA) and pure testosterone (WAKO, Japan). The anabolic to catabolic ratio was calculated as: (testosterone/cortisol) *1000.³⁰⁾

Statistical Analysis

We used a two-way ANOVA to evaluate the effects of experimental group and weeks into training on activity and hormone concentrations. We used the Student’s *t*-test (for normally distributed data) and Mann-Whitney’s *U* test (for skewed data) to compare the Osmopherine and placebo groups. Data are reported as mean $\pm$ SE.

Results

Activity

On days with exercise classes, the number of steps and energy expenditure of the men's activity significantly exceeded the of steps taken and the energy expenditure registered on other (rest) days (**Figure 1**). In the two-way ANOVA, we found that average daily activity (number of steps) and energy cost of activity (kcal/kg) registered during rest days (6 days between exercise days) did not depend on either the number of days into the training ($F_{4,39} = 0.45$, $P=0.77$; $F_{4,39} = 0.37$, $P=0.83$) or their pheromone exposure ($F_{1,39} = 0.96$, $P=0.33$; $F_{1,39} = 0.50$, $P=0.48$, correspondently). Further, neither factor (number of weeks into training or pheromone exposure) affected the number of steps or energy cost of activity ($F_{3,31} = 0.79$, $P=0.51$ and $F_{1,31} = 0.82$, $P=0.37$ for number of steps, and $F_{3,31} = 0.47$, $P=0.70$ and $F_{1,31} = 1.18$, $P=0.29$ for energy expenditure) on days with exercise classes.

Fig. 1. Diurnal activity (A) and energy expenditure from activity (B) during an exercise training course in older men exposed to placebo or Osmopherine.

Placebo Rest and Osmopherine Rest – average daily activity or energy expenditure during 6 days between exercise classes.

Placebo Exercise and Osmopherine Exercise - daily activity or energy expenditure on the days of exercise classes, including activity and energy expenditure recorded during class.

* $P \leq 0.05$ between day of the exercise class and rest days in the corresponding week (Student's paired t -test).

Testosterone

Using a two-way ANOVA, we calculated the influence of the date of sampling ($F_{4,39} = 3.05$, $P=0.029$) and pheromone exposure ($F_{1,39} = 1.86$, $P=0.18$) on the pre-exercise concentration of testosterone. Although there was no statistically significant effect of the Osmopherine application, the only increase in salivary testosterone during the training occurred in the group exposed to pheromone (**Figure 2**). Those subjects showed higher concentrations of testosterone compared with the baseline level (February 15), beginning from one week after Osmopherine application until the end of the study (March 15).

Fig. 2. Pre-exercise salivary testosterone in older men exposed to placebo or Osmopherine.

The concentration of testosterone in the saliva sampled after exercise did not differ from the pre-exercise levels (212 ± 4 pg/ml and 208 ± 5 pg/ml, respectively, $t=0.85$, $df=88$, $P=0.39$). The differences in the concentrations of testosterone in the post-exercise samples were caused by the number of weeks into training ($F_{4,39} = 5.84$, $P=0.001$) and did not depend on pheromone exposure ($F_{1,39} = 1.07$, $P=0.20$). In groups exposed to either Osmopherine or the placebo, the post-exercise testosterone levels exceeded the initial levels following the 2nd, 3rd, and 4th exercise classes (**Figure 3**).

Fig. 3. Post-exercise testosterone in saliva of older men exposed to placebo or Osmopherine.

Cortisol

We found no statistically significant difference in the concentrations of cortisol between samples collected before and after classes, perhaps because of the low intensity of exercise (5.94 ± 0.36 ng/ml and 6.18 ± 0.41 ng/ml, respectively, $t=0.44$, $df=88$, $P=0.66$). The two-way ANOVA showed that the number of weeks into the training course had an influence on the salivary cortisol levels in samples collected before and after exercise ($F_{4,35} = 4.39$, $P=0.006$ and $F_{4,39} = 2.89$, $P=0.036$, respectively). There was no significant effect of the pheromone on the concentration of cortisol in saliva samples collected before ($F_{1,39} = 1.71$, $P=0.20$) or after exercise ($F_{1,39} = 0.01$, $P=0.93$). In both groups, the concentrations of cortisol in saliva samples collected before and after exercise were significantly lower on the day of the last exercise class (March 15) than in the first day (February 15) of the training course (Figure 4, Figure 5).

Fig. 4. Pre-exercise cortisol in saliva of older men exposed to placebo or Osmopherine.

Fig. 5. Post-exercise cortisol in saliva of older men exposed to placebo or Osmopherine.

Testosterone to cortisol ratio (T/C ratio)

Both the number of weeks into the training course and pheromone exposure were statistically significant factors for the pre-exercise testosterone to cortisol ratio ($F_{4,39} = 12.0$, $P<0.001$ and $F_{1,39} = 4.8$, $P=0.035$, respectively). This ratio increased from the first to last exercise class (Figure 6). In samples collected before the 4th and 5th classes, the testosterone to cortisol ratio was significantly higher in the Osmopherine than in the Placebo groups. Two-ways ANOVA revealed a statistical significant effect of the number of weeks into the training course on the testosterone to cortisol ratio, assessed just after exercise classes ($F_{4,39} = 5.2$, $P=0.002$). But there was no a statistical significance effect of the pheromone exposure on the testosterone to cortisol ratio, assessed just after exercise classes ($F_{1,39} = 0.7$, $P=0.41$). As in post-exercise samples, this ratio increased from the first to the last exercise class in the group exposed to the placebo (Figure 7). In men exposed to Osmopherine, the testosterone to cortisol ratio in general rose after the 2nd class, then declined to its initial level and increased again after the last class.

Fig. 6. Pre-exercise testosterone to cortisol ratio of older men exposed to placebo or Osmopherine.
* $P=0.05$, ** $P = 0.01$ between placebo and Osmopherine groups (Student's t -test).

Fig. 7. Post-exercise testosterone to cortisol ratio of older men exposed to placebo or Osmopherine.
$P = 0.07$ between placebo and Osmopherine groups (Student's t -test).

Discussion

In this study, we examined the effects of female pheromone exposure and physical exercise on the anabolic and catabolic endocrine status in elderly Japanese men. This training program consisted of moderate exercise once a week. This level of exercise did lead to a statistically significant increase in the anabolic to catabolic balance in the older men due to opposite changes in levels of salivary testosterone and cortisol, especially in samples collected before exercise. The endocrine effect of regular exercise was more prominent when participants were exposed to a female pheromone along with training. The increased anabolic to catabolic ratio in men exposed to Osmopherine also resulted in a significant increase in testosterone and a decrease in cortisol.

The other hand, we were not able to observe the same changes from the post-exercise concentrations of testosterone and cortisol. The post-exercise testosterone to cortisol ratio declined at week 3 and week 4 in comparisons with week 2 in men exposed to Osmopherine. The cause of decline at week 3 and week 4 was the significant decrease of the cortisol in week 2. The low level of cortisol at week 2 provided the elevation of testosterone to cortisol ratio at week 2. It could be the effect of unpredictable response to the first application of Osmopherine or to other non-controllable factors.

The influence of female pheromones on the secretions from male gonads has been reported in several papers on animals, but in only a few studies on humans. Similar to our study, application of copulins (a female pheromone) was found to increase salivary testosterone in men.²⁵⁾ Another human pheromone, vomeropherin pregna-4,20-diene-3,6-dione (PDD), decreases serum LH and testosterone when delivered locally to the male human nasal cavity.³¹⁾ The opposite endocrine reactions of copulins and PDD could be a reflection of the different origins of these chemicals (sweat from the armpit and vaginal secretion).

Causal explanations for the endocrine effect of chemical signals are based on the perception of pheromones by the vomero-nasal organ (VNO), which projects into the hypothalamus.^{32,33)} Although the existence and olfactory function of the VNO in adults are still debated,²⁴⁾ several recent papers have reported VNO-related responses to sex pheromones in humans. For example, the electrophysiological response of VNO to nebulized sex pheromones was demonstrated in studies on humans,^{31,34)} and positron emission tomography was used to show that pheromones activate the hypothalamic area in healthy people.³⁵⁾ This effect occurs if female pheromones are presented to men, and male pheromones to women.

However, the subjects in those reports were significantly younger than the men in our experiment. Our finding that older men retain the capability to generate an endocrine response to female pheromones confirms the behavioral observations of Friebely and Rako.²⁶⁾ They reported that the addition of female pheromones to common perfume increased the sexual attraction of postmenopausal women. This effect was assessed by documenting behavioral changes in their contemporary partners.

In our study, we observed significant increase of testosterone by female pheromone exposure in the combination with exercise class in older men. Our data suggests the possibility of pheromone exposure to improve their anabolic to catabolic ratio. However, we did not evaluate their muscle mass and the sole effect of pheromone exposure. We need further researches to demonstrate the obvious effect of pheromone exposure on muscle atrophy.

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