The strength or weakness of respiratory muscles in comparison to other skeletal muscles due to their more specialized structures is considered as an indicator or cause of a disease. The most important of these diseases are specified as asthma, cystic fibrosis, neuromuscular diseases and chronic obstructive pulmonary disease (COPD). It is known that skeletal muscles increase their strength and endurance with appropriate load and resistance and undergo hypertrophy. Similarly, strength and endurance of respiratory muscles can also be increased like all other skeletal muscles. Inspiratory muscle training (IMT) is described as a remarkable exercise that exerts significant load on inspiratory muscles to strengthen the muscles of respiration. Respiratory muscle training has a rehabilitative effect as well. It is one of the primary methods used in pulmonary rehabilitation. Due to the strength-enhancing effect of respiratory muscle training on inspiratory muscle, it decreases the perception of dyspnea caused by decreased inspiratory muscle strength in COPD patients and provides an increase in exercise capacity. When studies conducted are examined, it can be seen that the positive effects of inspiratory muscle training have been determined on healthy.

Objectives: The aim of the present study is to investigate the effects of inspiratory muscle training and royal jelly supplementation on male reproductive hormones.

Methods: For this purpose, a total of forty male aged between 20-22 years were included in the study. Before the study, the groups who were addicted to smoking were divided into four groups as the control group (n: 10), royal jelly supplement group (n=10), IMT group (n=10), and royal jelly + IMT group (n=10). Blood samples were taken from all groups for analysis of LH, FSH and Testosterone levels. Maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) measurement for the groups to do training was carried out with 40% of their MIP values. Groups supplemented with royal jelly were provided with 1000 mg/day royal jelly supplied in glass vials. The training sessions were carried out at the same time every day for four weeks/five days.

Results: As a result of the study, it can be said that royal jelly supplement affects the reproductive hormones of smokers and increases the release of testosterone.

Conclusion: It can be said that training for the inspiratory muscles affects the mechanism of male reproductive hormones and can be used for rehabilitation in individuals who smoke.

Keywords: Exercise, royal jelly, respiration, reproductive, smoking.

Abstract

individuals,[6] individuals with lung disease,[7] healthy athletes,[8] obese individuals,[9] patients with hypertension,[10] elderly smoking addicts,[11] and healthy elderly individuals.[12] However, studies examining the effects of inspiratory muscle training on smoking addicts are limited, and the present study is important in that it is the first of its kind that investigates the effects of inspiratory muscle training on the reproductive system and the sex hormones secreted by it.

In the treatment of many diseases, the use of royal jelly is increasing day by day as a supplement to the medical treatment process.[13] Royal jelly is a dense milk product which young worker bees secrete from mandibular and hypopharyngeal glands and is used by to feed their larvae. [14] Queen bees are fed with royal jelly starting from the larvae period and royal jelly directly affects the life of the bees, allowing them to live up to five years by giving eggs as heavy as their weights each day.[15] It is recommended in order to minimize the damage caused by chemicals taken to the liver and kidneys and to protect these organs, especially in patients who use intensive antibiotics and receive radiotherapy and chemotherapy.[16] Due to these superior features, the use royal jelly as human food and its importance for human life and health are increasing more and more.[17] When the studies conducted are examined, it is seen that studies investigating the effects of royal jelly supplementation on fertility and reproductive functions in individuals who smoke are available in the literature, though limited in number.[18] However, there is no study investigating the effects of royal jelly supplementation along with inspiratory muscle training on sex hormones and mechanism in healthy smoking individuals. This study is the first of its kind that is believed to be contributing to the literature in terms of its method and the findings to be obtained.

In this study, it was hypothesized that inspiratory muscle training together with royal jelly supplementation would affect the reproductive system and hormones of smoking individuals. In the light of this information, what kind of effects inspiratory muscle training along with royal jelly supplementation will have on the reproductive system in smoking individuals has been the subject of the research.

Methods

Subjects

A total of 40 healthy male subjects volunteered to participate in this study (Table 1). The aim of the study was explained to all subjects and written informed consent was obtained from all subjects at the familiarization session. Before the study, the groups who were addicted to smoking were divided into by block randomization technique four groups as the control group (n=10), royal jelly supplement group (n=10), IMT group (n=10), and royal jelly + IMT group (n=10). Voluntary consent form was obtained from all participants in the study. In addition, permission to do the study was obtained from the Gaziantep University Clinical Research Ethics Committee.

Experimental Design

This study is a randomized, experimental study with a control group. The subjects visited the lab four times. During their first visit, all subjects were informed about the study and their descriptive information was recorded. First, by applying the Fagerström Test for Nicotine Dependence (FTND),[19] those with advanced degrees of dependence (6–7 points) and individuals who never smoked were divided into groups. During their second visit, blood samples were taken from all subjects prior to the study. During their third visit, the MIP and MEP values of individuals were determined and a nutrition program was given to all groups to establish a standard in nutrition. On their fourth visit, subjects performed the warm-up procedure with 40% of their MIP values for respiratory muscle training. The group given royal jelly supplement (1000 mg/day) and the groups performing IMT were invited to the laboratory at the same time (between 09:00 and 11:00 h.) 5 days a week.

Procedures

MIP and MEP Measurement

Electronic respiratory pressure meter was used to calculate MIP and MEP (Pocket Spiro MPM-100, Medical Electronic Construction R&D, Brussels, Belgium) according to the 2002 guidelines of the American Thoracic Society and European Respiratory Society.[20] Measurements were made using the

<table>
<thead>
<tr>
<th>Table 1. Descriptive information of subject (n=40)</th>
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<tbody>
<tr>
<td>Control Mean±SD</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Smoker Age (years)</td>
</tr>
<tr>
<td>Height (cm)</td>
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<tr>
<td>Weight (kg)</td>
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</tbody>
</table>
nasal plug in a sitting position. For MIP, the individual was asked to perform maximum inspiration against the closed respiratory tract and maintain it for 1-3 seconds. For MEP, the individual was asked to do maximum inspiration and was requested to perform maximum inspiration against the closed respiratory tract and maintain it for 1-3 seconds. The measurements were repeated between the 2 best findings until there was a 5% difference, and the average was recorded in cm H₂O.[21]

**Inspiratory Muscle Training Procedure**

A specific inspiratory training device (POWER®Breathe Classic, IMT Technologies Ltd., Birmingham; UK) was used for IMT. Training group subjects performed the IMT procedure at 40% of MIP (with +10% load increase each week and MIP test repeated on the first training day of every week). The IMT procedure included 30×2 dynamic inspiratory efforts (with 1 min interval) daily for 4 weeks.[22] A separate inspiratory muscle training device was used for each subject.

**Royal Jelly Supplement**

Royal jelly is seen as a beneficial natural food source for human metabolism and systems due to valuable substances contained in royal jelly content produced in the hypopharyngeal and mandibular glands of worker bees for the feeding of queen bees.[23] Royal jelly (Civan, Bee Farm, Bursa) was obtained in 1000 mg glass vials and was kept ready in the refrigerator. Groups supplemented with royal jelly received 1000 mg/day royal jelly in glass vials between 08.00 and 10.00 in the morning for four weeks.

**Blood Test Procedure**

Venous blood samples were collected from the right arm of the participants into 5ml at the central laboratory of Gaziantep University Faculty of Medicine between the hours of 09:00 and 10:00 in the morning a day prior to the study and the day after the study. At the end of the study, in order to analyze Luteinizing hormone (LH), Follicule Stimulating Hormone (FSH), Testosterone levels in the blood samples collected, serum samples obtained by centrifuging for 5 minutes at 4000 rpm in Nuve brand centrifuge device were studied in Beckman Coulter brand autoanalyser and the results were recorded.

**Statistical Analysis**

The SPSS version 22.0 (SPSS Inc., Chicago, IL) program was used for statistical analyzes. Values were represented as mean and standard deviation, and significance was set at 0.05. Kolmogorov-Smirnov test was performed to assess normality, and 2×4 mixed-factor analysis of variance and least significant difference tests were performed to analyze intra- and intergroup differences.

**Results**

Table 2 shows the analysis of pre- and post-tests. In the findings obtained at the end of the study, when LH and FSH levels were examined, a statistical significance was found in favor of post-test in the Royal Jelly (5.65±1.79), IMT (5.83±2.04) and RJ + IMT (5.14±1.06) group (p<0.05). When the difference between the groups in terms of LH and FSH levels was examined, a statistical significance was determined in the Royal Jelly (-1.43±1.48), IMT (-1.50±1.77) and RJ + IMT (-1.23±1.19) groups in comparison to the control groups (p<0.05). When testosterone levels were examined, a statistical significance was determined between pre-test and post-test results in favor of post-test in Royal Jelly (573.02±146.15), RJ + IMT

<table>
<thead>
<tr>
<th></th>
<th>Control Mean±SD</th>
<th>Royal Jelly Mean±SD</th>
<th>IMT Mean±SD</th>
<th>RJ+IMT Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH mIU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test</td>
<td>6.09±2.75</td>
<td>7.08±1.49</td>
<td>6.90±1.08</td>
<td>6.63±1.88</td>
</tr>
<tr>
<td>Post-test</td>
<td>5.86±2.07</td>
<td>5.65±1.79</td>
<td>5.83±2.04</td>
<td>5.14±1.06</td>
</tr>
<tr>
<td>Difference</td>
<td>-0.23±1.78</td>
<td>-1.43±1.48</td>
<td>-1.07±2.34</td>
<td>-1.49±1.15</td>
</tr>
<tr>
<td>FSH mIU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test</td>
<td>3.86±1.30</td>
<td>4.17±2.06</td>
<td>4.36±2.25</td>
<td>2.73±0.75</td>
</tr>
<tr>
<td>Post-test</td>
<td>3.79±1.43</td>
<td>3.85±1.86</td>
<td>3.93±1.60</td>
<td>2.44±0.55</td>
</tr>
<tr>
<td>Difference</td>
<td>-0.07±0.49</td>
<td>-0.32±0.33</td>
<td>-0.44±0.70</td>
<td>-0.29±0.34</td>
</tr>
<tr>
<td>Tests ng/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test</td>
<td>472.56±130.98</td>
<td>523.40±187.42</td>
<td>490.33±138.11</td>
<td>462.61±110.58</td>
</tr>
<tr>
<td>Post-test</td>
<td>478.64±189.28</td>
<td>573.02±146.15</td>
<td>503.63±139.34</td>
<td>508.16±94.61</td>
</tr>
<tr>
<td>Difference</td>
<td>6.06±80.29</td>
<td>49.62±42.27</td>
<td>13.30±78.89</td>
<td>45.54±45.71</td>
</tr>
</tbody>
</table>

S-CG: smoker control group, S-RJG: smoker royal jelly group, S-IMTG: smoker inspiratory muscle training group, S-IMT+RJG: smoker inspiratory muscle training with royal jelly group, A: significant difference between pre- and post-tests, B: significant difference from CG, C: significant difference from S-RJG, D: significant difference from S-IMTG, E: significant difference from S-IMT+RJG.
In our study, it was observed that royal jelly supplementation affects the reproductive system hormones in smoking individuals, but does not increase the secretion of testosterone hormone alone. Many studies have shown that respiratory muscle training has significant effects on respiratory muscles. It has been reported in several studies that the respiratory muscles will be stronger in a few days with respiratory muscle exercise, that the frequency of respiration decreases within three weeks, and that the performance increases as a result of the four-week respiratory muscle exercise. However, smoking causes functional disorders in the respiratory muscles by affecting the respiratory functions. The leading cause of the harmful effects of smoking on the respiratory system is the deterioration of the oxidant/antioxidant balance in smokers, inhibiting LH and FSH levels and increasing the level of testosterone hormone. The second finding is that the inspiratory muscle training affects the reproductive system hormones in smoking individuals, but does not increase the secretion of testosterone hormone alone.

There were 2 major findings of the present study: The first is that royal jelly supplement affects the reproductive system mechanism in smokers, inhibiting LH and FSH levels and increasing the level of testosterone hormone. The second finding is that the inspiratory muscle training affects the reproductive system hormones in smoking individuals, but does not increase the secretion of testosterone hormone alone.

Many studies have shown that respiratory muscle training has significant effects on respiratory muscles. It has been reported in several studies that the respiratory muscles will be stronger in a few days with respiratory muscle exercise, that the frequency of respiration decreases within three weeks, and that the performance increases as a result of the four-week respiratory muscle exercise. However, smoking causes functional disorders in the respiratory muscles by affecting the respiratory functions. The leading cause of the harmful effects of smoking on the respiratory system is the deterioration of the oxidant/antioxidant balance in favor of oxidants. In our study, it was observed that respiratory muscle training and royal jelly supplementation in smoking individuals inhibited LH and FSH levels in favor of post-test led to an increase in testosterone level. Many studies have shown that smoking has effects on sperm parameters, seminal plasma and various factors associated with infertility. Cigarette smoke contains gases, vaporized liquids and particles. During the burning of tobacco, approximately 4000 compounds are released due to chemical processes such as hydrogenation, pyrolysis, oxidation, decarboxylation and dehydration. Cigarette smoke contains many carcinogenic and mutagenic toxic chemicals such as nicotine and its metabolites, radioactive polonium, benzopyrene, dimethylnitrobenzanthracene, naphthalene, and polycyclic aromatic hydrocarbons. The major elements of smoking affecting the sperm parameters are Cadmium and Lead. The negative effect of cadmium on sperm parameters has also been observed in animal studies. Royal jelly has been used by humans in order to treat infertility as well as for its aphrodisiac effect from past to present. At the end of the study, it was found that when royal jelly and cyclosporin were used together, the toxic effects caused by cyclosporine alone, depending on the dose and time, decreased and it was suggested that royal jelly could be used as a preventive factor. Also, it was reported in a similar study that the amount of Glutathione Peroxidase enzyme decreased, that histopathological changes occurred in the prostate tissue, and that royal jelly prevented prostatic damage by reducing oxidative damage and apoptosis in the tissue.

Antioxidants, which are defense systems that combat these negative effects, protect lung cells from the damaging effects of oxidants. Harmful substances such as carbon monoxide, nitrogen oxide and hydrogen cyanide, nicotine and tar in cigarette smoke increase the production of free oxygen radicals such as singlet oxygen, superoxide, hydrogen peroxide and hydroxyl. Active or passive inhalation of cigarette smoke, exercise, stress and increased body temperature increase free oxygen radicals in the body, and these increased radicals increase the body’s need for vitamins. Cigarette smoke increases lipid peroxidation in the respiratory epithelium, increased free radicals resulting from cigarette smoking cause decreased lung volumes and capacities in the long run, and more importantly, lung cancer. It is known that respiratory exercises have positive effects on the lung system. The strengthening of the lung parameters depends on the performance of the respiratory muscles. In studies where the effects of royal jelly supplementation on reproductive hormones in individuals given royal jelly supplementation, its effects on fertility in the reproductive system in males, effects on testosterone and luteinizing hormone, antimicrobial, anticancer and immunomodulatory effects, and increasing effect on antioxidant activity have been revealed.

As a result, it can be said that royal jelly supplementation increases the testosterone release by stimulating the reproductive hormones of smokers. Inspiratory muscle training likewise affects the mechanism of male reproductive hormones. The royal jelly supplement along with respiratory muscle training has a healing effect on smoking individuals, prevents the formation of harmful radicals, positively affects the reproductive hormones by activating the antioxidant system and that it can be used for rehabilitative purposes in smoking individuals.

**Disclosures**

**Ethics Committee Approval:** The study protocol was approved by Gaziantep University Ethics Committee with 26/04/2017 dated and 168 numbered decision.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** None declared.
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