

## Research Article

# Effect of Inspiratory Muscle Training and Royal Jelly Supplement on Muscle Hypertrophy Markers in Male Smokers

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### Abstract

**Objectives:** Royal jelly (RJ) is one of the natural, valuable curative bee product due to its promising health-beneficial and nutritional properties. This healthy diet possesses anti-inflammatory, antimicrobial, antioxidants, antitumor, and immunomodulatory functions which benefit in humans health and welfare, resulting in its widespread medical use. The aim of this study is to investigate the effects of inspiratory muscle training and royal jelly supplementation on muscle hypertrophy markers of healthy smokers.

**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=0), IMT group (n=10), and royal jelly + IMT group (n=10). Blood samples were taken from all groups for analysis of LDH, CK, CK-MB, Cortisol and Testosterone levels. Groups were made to perform respiratory muscle training through inspiratory muscle training device 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:** In the findings obtained, a significance was found in the supplement group and the IMT group compared to the controls in the levels of LDH and Testosterone ( $p < 0.05$ ). A statistically significant difference was found in the LDH, CK and cortisol levels in IMT and supplement + IMT groups in favor of the posttest ( $p < 0.05$ ).

**Conclusion:** As a result, it was concluded that royal jelly supplementation increased LDH and testosterone levels by affecting the energy system, and inspiratory muscle training affected LDH, CK and cortisol levels.

**Keywords:** Hypertrophy, respiration, royal jelly, smoking

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In the treatment of many diseases, the use of royal jelly is increasing day by day as a supplement to the medical treatment process.<sup>[1]</sup> 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.<sup>[2]</sup> 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.<sup>[3]</sup> 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 radio-

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therapy and chemotherapy.<sup>[4]</sup> 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.<sup>[5]</sup> When the studies conducted are examined, studies on individuals who smoke royal jelly supplements are limited, but have been included in the literature.<sup>[6]</sup> However, there is no study investigating the effects of royal jelly supplements on muscle development and muscle hypertrophy in healthy and smokers with inspiratory muscle training. This study is the first study that is thought to contribute to science in terms of method and findings.

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).<sup>[7]</sup> It is known that skeletal muscles increase their strength and endurance with appropriate load and resistance and undergo hypertrophy.<sup>[8]</sup> Similarly, strength and endurance of respiratory muscles can also be increased like all other skeletal muscles.<sup>[9]</sup> Inspiratory muscle training (IMT) is described as a remarkable exercise that exerts significant load on inspiratory muscles to strengthen the muscles of respiration.<sup>[10]</sup> Respiratory muscle training has a rehabilitative effect as well. It is one of the primary methods used in pulmonary rehabilitation.<sup>[11]</sup> 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.<sup>[12]</sup> When studies conducted are examined, it can be seen that the positive effects of inspiratory muscle training have been determined on healthy individuals,<sup>[13]</sup> individuals with lung disease,<sup>[14]</sup> healthy athletes,<sup>[15]</sup> obese individuals,<sup>[16]</sup> patients with hypertension,<sup>[17]</sup> elderly smoking addicts,<sup>[18]</sup> and healthy elderly individuals.<sup>[19]</sup>

When the studies are examined, there is no study on royal jelly supplementation and inspiratory muscle training in individuals who smoke. This study is the first study that is thought to contribute to science in terms of method and findings. In this study, it was hypothesized that inspiratory muscle training together with royal jelly supplementation

would affect the muscle mechanism and create hypertrophy hormones in smokers individuals. In the light of this information, what kind of effects it will have on muscle hypertrophy and hormones has been the subject of research.

## Methods

### 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,<sup>[20]</sup> 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) 5 days a week. No groups were allowed to do exercise and high intensity physical activity.

### 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.

- 1<sup>st</sup> Group:** The Control (natural Sedentary, n=10) Group,
- 2<sup>nd</sup> Group:** The Royal Jelly supplement group which is took only Royal Jelly (RJ, n=10),
- 3<sup>rd</sup> Group:** Training Group which is inspiratory muscle training (IMT, n=10),
- 4<sup>th</sup> Group:** The Inspiratory muscle training which is supplied with royal jelly (RJ+IMT, n=10).

Before the study, the groups who were addicted to smoking were divided into four groups. Voluntary consent form

**Table 1.** Descriptive information of subject

	Control Mean±SD	Royal Jelly Mean±SD	IMT Mean±SD	RJ+IMT Mean±SD
(n=40)				
Age (years)	21.12±1.35	21.50±.75	22.12±1.12	20.75±.88
Height (cm)	177.87±6.24	176.12±5.02	177.12±4.88	175.75±7.06
Weight (kg)	76.25±15.99	75.62±4.40	76.75±10.36	71±9.60

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.

## 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.<sup>[21]</sup> Measurements were made using the nasal plug in a sitting position. For MIP, the individual was asked to perform maximum expiration and was requested 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 expiration 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 H<sub>2</sub>O.<sup>[22]</sup>

### 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.<sup>[23,24]</sup> 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.<sup>[25]</sup> 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 purple capped tubes 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 LDH, CK, Cortisol, CK-

MB and 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 2x4 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 LDH levels were examined, a statistical significance was found in favor of post-test in the smoking group Royal Jelly (208.67±58.89), IMT (193.50±38.59) and RJ+IMT (179.86±29.61) group.

When the difference between the groups in terms of LDH levels was examined, a statistical significance was determined in smoking individuals in the RJ (15.00±51.85) group, IMT (34.00±32.16) and RJ+IMT (21.86±24.54) groups in comparison to the control groups (p<0.05). As for CK levels, a statistical significance was identified in favor of the post-test in the smoking group in IMT (3.93±1.60) and RJ + IMT (2.44±0.55) groups (p<0.05). Considering the difference between the groups at CK levels, in both groups IMT and RJ + IMT groups; there was statistically significant difference between control groups and royal jelly groups (p<0.05). As for Cortisol levels, a statistical significance was identified in favor of the post-test in the smoking group in, IMT (10.24±4.20) and RJ + IMT (11.19±5.16) groups (p<0.05). When the difference between the groups in terms of Cortisol levels was examined, a statistical significance was determined in smoking individuals in the IMT (-2.87±4.83) and RJ + IMT (-2.30±4.95) groups in comparison to the control groups and royal Jelly (p <0.05). There was no statistically significant difference in CK-MB levels. As for Testosterone levels, a statistical significance was identified in favor of the post-test in the Royal Jelly, (573.02±146.15) and RJ + IMT (508.16±94.61) groups (p<0.05). When the difference between the groups in terms of Testosterone levels was examined, a statistical significance was determined in smoking individuals in the RJ (49.62±42.27) and RJ + IMT (45.54±45.71) groups in comparison to the control groups and IMT (p<0.05).

**Table 2.** Analysis of LDH, CK, CK-MB, Testosterone and Cortisol levels of smoker

	Control Mean±SD	Royal Jelly Mean±SD	IMT Mean±SD	RJ+IMT Mean±SD
LDH				
Pre-test	174.57±58.96	193.67±34.52	159.50±15.33	158.00±35.54
Post-test	177.71±18.13	208.67±58.89A	193.50±38.59 <sup>A</sup>	179.86±29.61 <sup>A</sup>
Difference	3.14±44.08	15.00±51.85B	34.00±32.16 <sup>BC</sup>	21.86±24.54 <sup>BC</sup>
CK				
Pre-test	180.71±32.02	190.33±56.81	128.00±40.31	172.00±67.18
Post-test	183.00±117.77	206.83±221.71	175.75±165.94 <sup>A</sup>	210.17±115.89 <sup>A</sup>
Difference	2.29±55.65	16.50±33.05	47.75±44.98 <sup>BC</sup>	38.17±85.24 <sup>BC</sup>
CK-MB				
Pre-test	2.43±0.95	1.89±1.24	1.42±0.50	2.05±1.28
Post-test	1.82±0.33	4.07±6.18	2.50±1.60	1.98±0.57
Difference	-0.62±0.76	2.18±5.36	1.08±1.25	-0.07±1.08
Cortisol				
Pre-test	11.30±1.98	9.53±1.86	13.10±4.49	13.49±3.37
Post-test	14.71±4.02	10.40±3.34	10.24±4.20 <sup>A</sup>	11.19±5.16 <sup>A</sup>
Difference	3.41±4.12	0.87±4.65	-2.87±4.83 <sup>BC</sup>	-2.30±4.95 <sup>BC</sup>
Tests				
Pre-test	472.56±130.98	523.40±187.42	490.33±138.11	462.61±110.58
Post-test	478.64±189.28	573.02±146.15 <sup>A</sup>	503.63±139.34	508.16±94.61 <sup>A</sup>
Difference	6.06±80.29	49.62±42.27 <sup>BD</sup>	13.30±78.89	45.54±45.71 <sup>BD</sup>

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 IMTG.

## Discussion

The aim of this study is to examine the effects of inspiratory muscle training and royal jelly supplementation on the biomarkers of muscle hypertrophy smoking individuals. There were 2 major findings of the present study; Royal jelly supplement affects the energy system mechanism, increasing LDH, creatine kinase and testosterone hormone level; It is the finding that inspiratory muscle training affects LDH, CK and cortisol values.

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.<sup>[26-28]</sup> 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.<sup>[29]</sup>

In our study, it was observed that respiratory muscle training increases lactate dehydrogenase and creatine kinase levels and decreases cortisol levels in smokers. LDH is a

type of enzyme used in the body to produce energy from sugar in energy metabolism. LDH produces lactate to provide stability in the tissues after the muscles work and high intensity exercises. In skeletal muscles with a high contraction rate, it turns the pyruvate into lactate, allowing the lactate to move from the muscle cell to the blood.<sup>[30]</sup> These changes in hormones during exercises are powerful stimulants for neuromuscular adaptations. These stimulants are the main factor in increasing general strength and power.<sup>[31]</sup> In addition, it is said that muscle hypertrophy is affected by variables such as exercise, nutrition, total testosterone, growth factors, growth hormone and cortisol. In our study, it was thought that respiratory muscle exercise changed these hormone levels due to the growth in muscles depending on the study. It has been conducted in studies that the muscle damage occurring in large muscle groups are performed such as warming, stretching, and massage,<sup>[32]</sup> but there are improvements in muscle damage with vitamin, mineral and food supplements.<sup>[33]</sup> 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.<sup>[34]</sup> Cigarette smoke contains many carcinogenic and mutagenic toxic chemicals such as nicotine and its metabolites,



radioactive polonium, benzopyrene, dimethylbenzanthracene, naphthalene, and polycyclic aromatic hydrocarbons.<sup>[35,36]</sup> These toxic substances negatively affect muscle growth and development by damaging muscle cells and tissues. It can be said that in cases of stress created by exercise, IMT decreases cortisol level and affects the healing of muscles.

Royal jelly has been used by humans for many purposes including cancer, as well as an aphrodisiac effect from past to present. Teixeira et al. suggested that royal jelly decreased lipid peroxidation in the cerebral cortex and striatum, and strengthened the glutathione defense system in the cerebral cortex and striatum, in a study with rats that were kept in a narrow area and exposed to cold. It has also been shown to reduce the level of corticosterone by strengthening the brain antioxidant system and suggested that royal jelly can be used as an anti-stress agent.<sup>[37]</sup> Antioxidants, which are defense systems that combat these negative effects, protect lung cells from the damaging effects of oxidants.<sup>[38]</sup> 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.<sup>[39]</sup> 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.<sup>[40]</sup> Cigarette smoke increases lipid peroxidation in the respiratory epithelium,<sup>[41]</sup> increased free radicals resulting from cigarette smoking cause decreased lung volumes and capacities in the long run,<sup>[42]</sup> respiratory and circulatory system diseases,<sup>[43]</sup> and more importantly, lung cancer.<sup>[44]</sup> It is known that respiratory exercises have positive effects on the lung system.<sup>[45]</sup> The strengthening of the lung parameters depends on the performance of the respiratory muscles.<sup>[46]</sup>

Consequently, royal jelly supplements and inspiratory muscle exercises revealed the positive effects of exercise on muscle tissue in smokers. At the end of the study, it is concluded that a healing and enhancing muscle by affecting muscle hypertrophy. It can also be said that by activating the healing, antioxidant system, it can be used for rehabilitation purposes in individuals who smoke and develop enzymes positively.

#### Disclosures

**Ethics Committee Approval:** The study protocol was approved by Gaziantep University Ethics Committee with 26/04/2017 dated and 311 numbered decision.

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

**Conflict of Interest:** None declared.

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