

# Effect of Inspiratory Muscle Training with Royal Jelly Supplement on Iron Metabolism in Cigarette Addicts

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## ABSTRACT

**Objective:** The strength or weakness of the respiratory muscles compared with other skeletal muscles owing to their more specialized structures is considered as an indicator or cause of a disease. This study aimed to investigate the effects of inspiratory muscle training with royal jelly (RJ) supplement on iron metabolism in cigarette addicts.

**Materials and Methods:** A total of 40 male volunteers participated in the study who were divided into 4 groups before the study as follows: smoker control group (n=10), inspiratory muscle training (IMT) group (n=10), RJ group (n=10), and RJ+IMT (n=10) group. Blood samples were taken from all the participants for analysis of iron, iron binding, and total iron binding capacity. Maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) measurements for the groups to perform training were carried out with 40% of their MIP values. The training sessions were carried out at the same time every day for 4 weeks (5 days per week).

**Results:** The Statistical Package for Social Sciences version 22.0 program was used for statistical analyses. The results of the analysis found that the iron, iron binding, and total iron binding capacities were in favor of post-tests in the RJ supplement IMT, and RJ+IMT groups compared with those in the control group ( $p<0.05$ ). There was a statistically significant difference between the RJ supplement, IMT, and RJ+IMT groups compared with the control group ( $p<0.05$ ).

**Conclusion:** Therefore, it could be concluded that the IMT and RJ supplements positively affected these parameters by altering the iron metabolism of the cigarette addicts.

**Keywords:** Respiratory circulation, nicotine addiction, food supplementations

## Introduction

The use of royal jelly (RJ) is increasing by the day as a supplement in the medical treatment process [1]. RJ is a dense milk product, which young worker bees secrete from the mandibular and hypopharyngeal glands, and is used by to feed their larvae [2]. The queen bees are fed with RJ starting from the larvae period, and RJ directly affects the life of the bees, allowing them to live up to 5 years by laying eggs each day that are as heavy as their own bodyweight [3]. RJ is recommended in patients, who use intensive antibiotics and receive radiotherapy and chemotherapy, to minimize the damage caused by chemicals to the liver and kidneys and to protect these organs [4]. Owing to these superior features, the use of RJ as human food and its importance in human life and health is increasing [5].

Smoking is one of the leading causes of preventable mortality and morbidity in our country. The negative effects of smoking on human health depend on the various substances present in cigarette smoke [6]. The most important of the harmful effects of smoking are asthma, cystic fibrosis, neuromuscular diseases, and chronic obstructive pulmonary disease [7]. The strength or weakness of the respiratory muscles compared with other skeletal muscles owing to their more specialized structures is considered as an indicator or cause of a disease. Similarly, strength and endurance of the respiratory muscles can also be increased like other skeletal muscles [8]. Inspiratory muscle training (IMT) is a remarkable exercise that exerts a significant load on inspiratory muscles and strengthens the muscles of respiration [9]. Respiratory muscle training

has a rehabilitative effect as well. It is one of the primary methods used in pulmonary rehabilitation [10]. Because of the strength-enhancing effect of respiratory muscle training on inspiratory muscle, it decreases the perception of dyspnea caused by decreased inspiratory muscle strength in patients with chronic obstructive pulmonary disease and provides an increase in exercise capacity [11]. Studies have shown the positive effects of IMT on healthy individuals [12], those with lung disease [13], healthy athletes [14], obese individuals [15], patients with hypertension [16], elderly cigarette addicts [17], and healthy elderly individuals [18].

However, studies on IMT are rare in smokers. Therefore, this study aimed to examine the effects of IMT and RJ supplementation on iron metabolism in cigarette addicts. This is the first study to contribute to science in terms of the effects of inspiratory muscle exercises on iron metabolism, describing the methods and findings.

## Materials and Methods

### Experimental Design

This study is a randomized experimental study with a control group. The participants visited the laboratory 4 times. During their first visit, all participants were informed about the study and their descriptive information was recorded (Table 1).

By applying the Fagerström test for nicotine dependence [19], participants with advanced degrees of dependence (6–7 points) and those who never smoked were divided into groups. During their second visit, blood samples were taken from all participants before the study. During their third visit, the maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) values of the individuals were determined, and a nutrition program was given to all groups to establish a standard in nutrition. On their fourth visit, the participants performed the warm-up procedure with 40% of their MIP values for respiratory muscle training. The groups performing IMT were invited to the laboratory at the same time (between 9:00 and 11:00) 5 days a week. No groups were allowed to do exercise and high-intensity physical activity.

### Main Points

- Inspiratory muscle exercises positively affect iron metabolism in smokers.
- Royal jelly supplement positively affects the iron metabolism of individuals who smoke.
- It is thought that breathing exercises and royal jelly supplement can increase oxygen capacity by affecting iron metabolism in smokers.

**Table 1.** Descriptive information of the participants

N=40	C Mean±SD	RJ Mean±SD	IMT Mean±SD	RJ+IMT Mean±SD
Age (years)	21.12±1.35	21.50±0.75	22.12±1.12	20.75±0.88
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

CG: control group; RJG: royal jelly supplement group; IMT: inspiratory muscle training group; RJ+IMTG: smoking inspiratory muscle training+royal jelly supplement group; SD: standard deviation

**Table 2.** Analysis of pre- and post-tests

		CG Mean±SD	RJG Mean±SD	IMTG Mean±SD	RJ+IMTG Mean±SD
Iron	Pre-test	102.71±35.41	98.67±13.60	123.50±70.43	125.14±15.16
	Post-test	101.43±46.79	120.00±60.53 <sup>A</sup>	157.75±53.68 <sup>A</sup>	164.43±24.62 <sup>A</sup>
	Difference	-1.29±44.05	21.33±48.78 <sup>B</sup>	34.25±96.04 <sup>B</sup>	39.29±28.32 <sup>B</sup>
Iron Binding	Pre-test	255.00±66.77	223.83±88.61	212.88±64.49	277.86±44.20
	Post-test	260.57±73.89	211.67±26.33 <sup>A</sup>	187.50±72.32 <sup>A</sup>	263.14±43.40 <sup>A</sup>
	Difference	5.57±58.33	-12.17±92.02 <sup>B</sup>	-25.38±66.49 <sup>B</sup>	-14.71±43.61 <sup>B</sup>
T.I.B.	Pre-test	358.14±35.22	355.50±27.70	371.63±37.61	352.71±32.34
	Post-test	364.86±19.05	331.83±46.72 <sup>A</sup>	345.25±41.50 <sup>A</sup>	333.00±48.96 <sup>A</sup>
	Difference	6.71±22.12	-23.67±26.39 <sup>B</sup>	-26.38±13.94 <sup>B</sup>	-19.71±22.37 <sup>B</sup>

CG: control group, RJG: royal jelly supplements group, IMTG: inspiratory muscle training group, A: significant difference between pre- and post-tests, B: significant difference from CG

### Subjects

A total of 40 healthy men aged 21–22 years voluntarily participated in the study (Table 1). The groups were made by the block randomization technique. In this method, the number of groups and probabilities in each block must be equal. When there are 2 groups (A and B), the size of the block should be 2, 4, 6, and so on; when there are 3 groups (A, B, and C), the sizes should be 3, 6, 9, and so on. In practice, the number of participants in the block is 4, rather small, whereas the ideal is to have 6 to 12 participants in the blocks. Because it is easy to use, the Excel package program (Microsoft Office 2007, version 16.0, Microsoft Corp. Redmond, WA, ABD) has been preferred in applications [20].

The aim of the study was explained to all the participants, and voluntary written informed consent was obtained from them at the familiarization session. In addition, permission to conduct the study was obtained from the Gaziantep University clinical research ethics committee. Before the study, the participants who were addicted to smoking were divided into 4 groups:

1. The control (natural sedentary, n=10) group
2. The RJ supplement group, who only took

RJ (n=10)

3. The IMT group (IMT, n=10)
4. The IMT group who took RJ (RJ+IMT, n=10).

### Procedures

#### Maximal Inspiratory Pressure and Maximal Expiratory Pressure Measurements

Electronic respiratory pressure meter (Pocket Spiro MPM-100, Medical Electronic Construction R&D, Brussels, Belgium) was used to calculate the MIP and MEP according to the 2002 guidelines of the American Thoracic Society and European Respiratory Society [21]. Measurements were made using the nasal plug in a sitting position. For MIP, the individual was asked to perform maximum expiration and maximum inspiration against the closed respiratory tract and maintain it for 1–3 seconds. For MEP, the individual was asked to perform maximum inspiration and 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 cmH<sub>2</sub>O [22].

#### Inspiratory Muscle Training Procedure

A specific inspiratory training device (POWER@Breathe Classic, IMT Technologies Ltd., Birmingham; UK) was used for IMT. The

training group participants 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 minute interval) daily for 4 weeks [23, 24]. A separate IMT device was used for each participant.

### Royal Jelly Supplement

RJ (Civan, Bee Farm, Bursa) was obtained in 1,000 mg glass vials and kept ready in the refrigerator. The groups supplemented with RJ received 1,000 mg/day of RJ in glass vials between 8.00 and 10.00 in the morning for 4 weeks.

### Blood Test Procedure

Venous blood samples were collected from the right arm of the participants into 5-mL purple capped tubes at the central laboratory of Gaziantep University Faculty of Medicine between 9:00 and 10:00 in the morning on the day before and the day after the study. At the end of the study, to determine iron, iron binding, and total iron binding levels in the collected blood samples, the blood samples taken at the end of the study were analyzed automatically on the Beckman Coulter LH 780 instrument.

### Statistical Analysis

The Statistical Package for Social Sciences software version 22.0 (IBM SPSS Corp.; Armonk, NY, USA) program was used for statistical analysis. Values were represented as mean and standard deviation, and significance was set at 0.05. The 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 inter-group differences. The study protocol was approved by the Gaziantep University ethics committee dated 26/04/2017 and numbered 168.

### Results

The results of the analysis are shown in Table 2. There was a statistically significant difference in the iron, iron binding, and total iron binding capacities post-tests in the RJ supplement, IMT, and RJ+IMT groups compared with the control group ( $p < 0.05$ ). There was also a statistically significant difference between the groups in the RJ supplement, IMT, and RJ+IMT groups compared with their controls ( $p < 0.05$ ).

### Discussion

This study aimed to investigate the effects of IMT and RJ supplementation on iron metabolism in individuals addicted to cigarettes. The results demonstrated that these applications had positive effects in regulating iron metabo-

lism in groups receiving RJ supplements, IMT, and RJ+IMT.

Multiple studies have shown that respiratory muscle training has a significant effect on the respiratory muscles. It has been reported in several studies that the respiratory muscles become stronger in a few days with respiratory muscle exercise, the frequency of respiration decreases within 3 weeks, and the performance increases as a result of the 4-week respiratory muscle exercise [25-27]. Iron is an essential element necessary for all the cells. Its most important task is to carry oxygen through hemoglobin. Iron catabolizes oxygenation, hydroxylation, and various other metabolic events [28]. IMT stimulated iron metabolism in the study group compared with the control groups, resulting in a significant change in iron, iron binding, and total iron binding capacity. This is thought to be related to the increase in oxygen carrying capacity of the myoglobin in the muscle because of the work of the muscles.

Iron is an essential element because it is necessary for erythropoietic function, oxidative metabolism and cellular immunity. The total amount of iron in the body for an adult man is 3,500 mg (50 mg/kg). Most of the iron in the body is distributed within hemoglobin (65%; 2,300 mg). Approximately 10% (350 mg) is present in the muscle fibers (myoglobin) and other tissues, such as enzymes and cytochromes [29]. 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 [30]. Cigarette smoke contains gases, vaporized liquids, and particles. The burning of tobacco releases approximately 4,000 compounds because of chemical processes, such as hydrogenation, pyrolysis, oxidation, decarboxylation, and dehydration [31]. In our study, it was observed that respiratory muscle training and RJ supplementation in individuals who smoked cigarettes decreased iron, iron binding and total iron binding levels post-test. Cigarette smoke contains many carcinogenic and mutagenic toxic chemicals, such as nicotine and its metabolites, radioactive polonium, benzo[a]pyrene, dimethylbenzanthracene, naphthalene, and polycyclic aromatic hydrocarbons [32, 33]. These toxic substances negatively affect the muscle growth and development by damaging the muscle cells and tissues. Harmful substances, such as carbon monoxide, nitrogen oxide, hydrogen cyanide, nicotine and tar, in cigarette smoke increase the production of free oxygen radicals, such as singlet oxygen, superoxide,

hydrogen peroxide, and hydroxyl [34]. Cigarette smoke increases lipid peroxidation in the respiratory epithelium [35]; the increased free radicals from cigarette smoking cause decreased lung volumes and capacities in the long term [36], respiratory and circulatory system diseases and most importantly, lung cancer. It is known that respiratory exercises have positive effects on the lung system. Strengthening of the lung parameters depends on the performance of the respiratory muscles [37].

Antioxidants, which are defense systems that combat these negative effects, protect the lung cells from the damaging effects of oxidants. Active or passive inhalation of cigarette smoke, exercise, stress, and increased body temperature increase the free oxygen radicals in the body, which then increase the body's need for vitamins [38].

In conclusion, IMT and RJ supplement in the respective groups stimulated iron metabolism compared with that in the control groups, resulting in a significant change in iron, iron binding, and total iron binding capacity. This could be owing to the increase of oxygen carrying capacity of myoglobin in the muscle because of the work of the muscles.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of the Gaziantep University (26.04.2017 / 168).

**Informed Consent:** Informed consent was obtained from patients who participated in this study.

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

**Conflict of Interest:** The author has no conflict of interest to declare.

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