



# Effects of TABATA Exercise Volume and Royal Jelly Supplementation on NLRP3 Inflammasome and lncRNA-H19 Expression in Obese Men

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## Abstract

**Background:** Both coding and long non-coding RNAs (lncRNAs) have emerged as vital regulators in almost every cellular process, and their expression can be modulated by external stimuli, such as physical exercise.

**Objectives:** The current research aimed to investigate the effects of different volumes of TABATA-high-intensity interval training (HIIT) exercises combined with royal jelly (RJ) supplementation on the NLRP3 inflammasome and lncRNA-H19 expression in obese males.

**Methods:** Forty-two healthy men [Body Mass Index (BMI) = 30 kg/m<sup>2</sup>, waist-to-hip ratio = 0.95, age range: 40 - 60 years] volunteered to participate in the study. The individuals were randomly divided into five experimental groups (N = 35) and one control + placebo group (N = 7). The high-volume (HV) or low-volume (LV) TABATA exercise programs were performed twice a week for 8 weeks. Participants in the RJ supplementation groups received a 1000 mg capsule once a day for 8 weeks. The expression of NLRP3 and lncRNA-H19 genes was evaluated using the real-time PCR method.

**Results:** The NLRP3 gene expression in the Bruce test, measured before and after the 8-week exercise interventions and RJ supplementation, showed insignificant changes across the different groups. However, the H19 gene expression in the Bruce test showed a significant reduction in the HV-TABATA HIIT intervention groups, which was more pronounced than in the LV groups after 8 weeks: HV group (P = 0.004), RJ group (P = 0.001), HV + RJ group (P = 0.007), and LV + RJ group (P = 0.002). After 8 weeks of non-pharmacological interventions involving exercise training and supplementation, a significant decrease in NLRP3 and a significant increase in H19 gene expression were detected in the HV group compared to the LV group (P = 0.05 and P = 0.010). Significant improvement was also found in the resting H19 levels between the RJ and LV groups (P = 0.011) and the LV + RJ group (P = 0.44). Moreover, a significant reduction in resting NLRP3 gene expression was observed between the RJ + LV and LV groups (P = 0.038).

**Conclusions:** Chronic HV TABATA HIIT exercise, when combined with RJ supplementation, is effective in attenuating inflammatory responses to acute stress.

**Keywords:** Inflammasome, Exercise Trainig, Food Supplement, Gene Expression

## 1. Background

According to the World Health Organization (WHO), obesity is characterized by an excessive and unhealthy buildup of body fat, which is associated with a more pro-inflammatory cytokine circulation profile (1). The quarantine during the COVID-19 pandemic, enforced in many populations, led to an increase in risk factors related to physical inactivity, such as weight gain,

obesity, low-grade inflammation, elevated blood pressure, insulin resistance, and mental health issues (1). It is clear that alternative strategies are needed for maintaining health during public health crises similar to the COVID-19 pandemic (2).

Long non-coding RNAs (lncRNAs), which are non-protein-coding transcripts exceeding 200 nucleotides in length, play a vital role in regulating gene expression. Aberrant expression of lncRNAs is strongly associated

with lipid accumulation and cardiovascular (CV) problems (3). Recently, it has been shown that lncRNAs are involved in adipogenesis, which has implications for adult obesity and obesity-related problems (1). Furthermore, evidence of obesity-related disorders in CV diseases highlights the production of pro-inflammatory cytokines, mediated by the activation of inflammasomes (4). Among these inflammasomes, the most intensively studied is the NOD-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome. Dysregulated activation of the NLRP3 inflammasome is implicated in the etiology of many disorders, including systemic inflammation and CV diseases (5). There is evidence that NLRP3 inflammasome activation is tightly regulated by various mechanisms, particularly post-transcriptional modulation via microRNAs (6). Among the long list of genes encoding lncRNAs, H19 is an lncRNA whose expression is restricted to adipose tissue and is involved in obesity-related conditions (3). Recently, a growing number of studies have revealed that H19 expression declines with increasing Body Mass Index (BMI) in obese individuals, suggesting that the expression of this gene may help prevent the progression of dietary obesity (3). Understanding the precise role of lncRNAs in controlling the activity of the NLRP3 inflammasome can aid in designing conventional non-pharmacological strategies, such as lifestyle modifications, for individuals with obesity and multiple inflammatory diseases. While there is still much to discover in terms of ideal treatments, non-pharmacological strategies aimed at managing obesity during physical inactivity induced by COVID-19 are emerging.

Before the COVID-19 outbreak, retrospective studies strongly suggested that regular exercise training was associated with a lower risk of CV problems (7). Numerous studies indicate that high-intensity interval training (HIIT) may be an effective tool for achieving desired changes in health-related fitness in the general population, as well as for preventing chronic lifestyle diseases such as obesity, metabolic syndrome, and type 2 diabetes (8). While there is substantial evidence confirming the effects of HIIT exercises on CV health markers in adults with overweight/obesity (9), the difficulty in prescribing an adequate dose of HIIT for CV health is largely due to the variability in protocols used in interventions. One method of HIIT workout or interval training at high intensity is the TABATA training method (10), first introduced by Japanese scientist Izumi TABATA in 1996 (10). This protocol can be easily controlled and performed without the need for complicated equipment, and it can be adapted to different environments, including domestic settings, in

a time-efficient manner. It aims to prevent detraining and even improve physical fitness and general health (10). Cowan et al. examined the separate effects of exercise amount and intensity on adipose tissue and skeletal muscle mass in adults with abdominal obesity. They concluded that adult groups on a low-intensity, low-volume (LV) exercise regimen, who exercised for 30 minutes per session, showed similar reductions in mass compared to groups with high-volume (HV), high-intensity training, as well as those with HV, low-intensity training (11). An increasing number of studies are pursuing effective pharmacological and non-pharmacological strategies for controlling inflammation and obesity (6). However, the role of non-pharmacological strategies, such as LV and HV TABATA-HIIT exercises—particularly when paired with herbal supplementation—on coding and non-coding genes such as NLRP3 and lncRNA-H19, remains unclear. These studies may guide future investigations into the physiological mechanisms underlying the benefits observed in previous HIIT trials.

In recent years, the consideration of natural products as anti-inflammatory and antioxidant treatments has grown worldwide (12). Royal jelly (RJ) is a yellowish-white, creamy, and acidic natural product secreted from the hypopharyngeal and mandibular glands of nurse honeybees (*Apis mellifera*). It is now widely used as a dietary supplement to improve human health (12). The importance of RJ has attracted global attention, as evidenced by the increasing number of publications and citations in the Web of Science core collection (13). The literature has consistently highlighted RJ's biological activities, including antimicrobial, antitumor, hepatoprotective, immunomodulatory, anti-hypercholesterolemic, antioxidant, and antidiabetic properties (12). Additionally, RJ administration has been shown to successfully inhibit the production of pro-inflammatory cytokines without exerting cytotoxic effects on macrophages *in vitro* (13). However, research gaps remain in the knowledge and literature regarding the effects of RJ supplements on the encoding genes of the NLRP3 inflammasome and lncRNA-H19, particularly when combined with low- and HV TABATA-HIIT exercises, warranting further investigation.

Given that the effects of individual and combined LV and HV TABATA-HIIT exercises paired with RJ supplementation on coding (NLRP3) and long non-coding (lncRNA-H19) genes have not been explored in the literature, it was hypothesized that 8 weeks of RJ supplementation, along with low- and HV TABATA-HIIT exercises, may improve lncRNA-H19 gene expression by decreasing the inflammasome NLRP3.

## 2. Objectives

Therefore, the purpose of this study was to investigate the chronic 8-week effects of low- and HV TABATA-HIIT exercises, with and without RJ supplementation, on NLRP3 and lncRNA-H19 expression in obese men.

## 3. Materials and Methods

### 3.1. Study Design, Participants, and Ethical Compliance

The institutional ethics committee approved the current study (ethical code: [IR.UMZ.REC.1401.022](#)), with protocols closely following the most recent iteration of the Declaration of Helsinki. All participants provided informed consent, which included the freedom to object to any aspect of the research protocol and the right to withdraw from the experiment at any time without incurring any fees.

Forty-two healthy men between the ages of 40 and 60 volunteered to take part in the study. Participants were randomly allocated into six groups using a lottery method (each member of the community was assigned a unique code or number): Obese control with placebo (OBC), high-volume HIIT (HV-HIIT), low-volume HIIT (LV-HIIT), RJ, high-volume HIIT with RJ (HV-HIIT + RJ), and low-volume HIIT with RJ (LV-HIIT + RJ). Before the study began, all participants were familiarized with the testing protocols, equipment, and procedures. Each group underwent four tests to evaluate the expression of the lncRNA-H19 and NLRP3 inflammasome genes. Tests were conducted before and after the Bruce exercise protocols, at the start and end of the eight-week low- and HV TABATA-HIIT exercise interventions. The test results were compared between groups with and without RJ supplementation interventions.

The present study protocol was carried out during the COVID-19 pandemic. Consequently, the exclusion criteria included the following: The presence of clinical features not explained by any other illness; a history of travel to another nation or state within 14 days prior to the experiment; at least one sign or symptom of fever or acute respiratory disease (such as cough or respiratory distress); or close contact with a patient confirmed positive for SARS-CoV-2 by an RT-PCR test. It should be noted that, to rule out the possibility of "silent hypoxemia," also referred to as "happy hypoxia," all participants underwent pulse oximeter testing to ensure that their arterial oxygenation levels were within the normal range ( $SpO_2 \geq 95\%$ ) (14). Additional inclusion criteria included a hemoglobin (HGB) level of  $\geq 11$  g/dL

and the absence of hemoglobinopathies, such as thalassemia, that could impair the blood's oxygen-carrying capacity (15). Similarly, participants were excluded from the study if they completed fewer than 12 of the 16 scheduled exercise sessions. Inclusion in the study was limited to individuals without a history of smoking and without signs or symptoms of chronic cardiac, pulmonary, or inflammatory diseases, as well as any other medical contraindications.

### 3.2. Anthropometric Measurements

Anthropometric measurements were taken in the morning during the participants' initial visit to the laboratory. A certified medical scale equipped with a stadiometer was used to measure body mass and height. A body composition analyzer (Medigate Inc., Boca X1, Korea) was also used to calculate the waist-to-hip ratio (WHR) and BMI. [Table 1](#) provides an overview of the participants' demographic details. Additionally, a questionnaire was administered to record the participants' medical and physical activity history. This was followed by a physical examination and vital assessments, including spirometry, blood pressure, heart rate (HR), and a resting electrocardiogram.

### 3.3. The Maximal Exercise Protocol (Bruce Test)

Before the exercise test, all participants received an explanation of the experimental procedures. Standard environmental parameters were maintained during data collection: 24 - 26°C, 760 mmHg barometric pressure, and 50 - 60% relative humidity. The specifics of the maximal exercise regimen (Bruce test) used in this investigation have been previously published (16). In summary, participants followed the experimental protocol, which was based on a percentage of maximum HR that was determined during a preliminary visit. Prior to initiating the protocol, HR was continuously measured using a Custo med GmbH ECG system (XP, Vista, Leibnizstraße 2000. 7. Ottobrunn, 85521, Germany). To improve signal detection, participants wore a Polar HR transmitter belt (Polar FS1, Polar Electro, OY, Finland) around their chest. The operator closely monitored the participants throughout the maximal exercise protocol, recording systolic blood pressure (SBP), HR, any chest pain, and any changes in the ECG at each stage, as well as during recovery for three to five minutes after the exercise ended. The exercise electrocardiography was stopped when participants' HRs reached 80 - 90% of the maximum HR anticipated for HIPE. Additional guidelines for terminating the exercise electrocardiography test were based on the 2002 revision of the exercise testing guidelines

**Table 1.** Demographic and Body Composition Characteristics of the Participants <sup>a</sup>

Groups	OBC (N = 7)	RJ (N = 6)	HF-HIIT (N = 7)	LF-HIIT (N = 8)	HF-HIIT + RJ (N = 7)	LF-HIIT + RJ (N = 7)	F	P-Value
Age, y	48 ± 3	46 ± 4	45 ± 5	47 ± 8	49 ± 6	49 ± 4	0.44	0.81
Weight, kg	105 ± 16	86 ± 10	89 ± 13	90 ± 13	91 ± 17	89 ± 13	1.49	0.22
BMI, kg/m <sup>2</sup>	31.6 ± 3.6	30.4 ± 3	30 ± 3.6	30.7 ± 3.6	30 ± 4	29.6 ± 3.9	0.31	0.90
PBF, %	29.1 ± 6	31.4 ± 2	29.6 ± 4	30.6 ± 5	26.7 ± 3	28.7 ± 5	0.96	0.46
WHR	0.96 ± 0.06	0.92 ± 0.04	0.95 ± 0.03	0.94 ± 0.04	0.95 ± 0.04	0.94 ± 0.03	0.83	0.53
AVI, cm <sup>2</sup>	22 ± 4	18 ± 3	20 ± 2	20 ± 2	19 ± 3	19 ± 2	0.63	0.68
CI m <sup>2/3</sup> /kg <sup>1/2</sup>	1.28 ± 0.04	1.23 ± 0.027	1.28 ± 0.05	1.26 ± 0.05	1.26 ± 0.03	1.26 ± 0.04	1.30	0.29
Peak power, Watt	1378 ± 218	1135 ± 308	1254 ± 34	1121 ± 252	1209 ± 332	1129 ± 397	0.73	0.61

Abbreviations: OBC, obesity control; HV-HIIT, high-volume HIIT; LV-HIIT, low-volume HIIT; RJ, royal jelly; HV-HIIT + RJ, high-volume HIIT + royal jelly; LV-HIIT + RJ, low-volume HIIT + royal jelly; BMI, Body Mass Index; VO<sub>2max</sub>, maximal oxygen consumption; WHR, waist-to-hip ratio; AVI, Abdominal Volume Index; CI, Conicity Index.

<sup>a</sup> Values are expressed as mean ± SD.

published by the American College of Cardiology (ACC) and the American Heart Association (AHA) (17).

#### 3.4. The TABATA-High-Intensity Interval Training Exercise Program

Each participant received an explanation of the specifics of the HV and LV TABATA-HIIT exercise programs during the preparatory and practice sessions. The body weight-based exercise training program was modified from earlier research with a few adjustments while maintaining the overload principle, which involves progressively increasing sets, exercise complexity, and the RPE Scale (18). For eight weeks, participants engaged in the exercise training program twice a week. In summary, the training sessions started with two sets and concluded with four sets. Additionally, the HV-HIIT exercise program consisted of eight sequences per set, each involving 20 seconds of work followed by 10 seconds of rest (ratio 2:1), performed over four minutes (Tabata model). In contrast, the LV-HIIT exercise program lasted five minutes and involved eight sequences of 20 seconds of work followed by 20 seconds of rest (ratio 1:1).

In the LV-HIIT program, each exercise movement lasted 15 seconds in the first and third weeks and 20 seconds in the second and fourth weeks. In the HV-HIIT program, exercise movements lasted 20 seconds in the first week, 25 seconds in the second and third weeks, and 30 seconds in the fourth week (Table 2). The predicted maximal HR (220 - age) was used to determine exercise intensity, which ranged between 80 and 90 percent of the maximum HR (170 - 180 bpm). Each workout session included a 10-minute warm-up and a five-minute cool-down. There was a 60-second recovery period between each set and exercise movement.

#### 3.5. Royal Jelly Supplementation Intervention

The RJ supplementation protocol was designed based on the results of previous studies (12). Accordingly, each individual in the RJ groups received 24 supplements, each containing a 1000 mg dose, for eight weeks. The 1000 mg RJ used in this study was imported from Canadian Organic Health Products. Participants were instructed to consume the supplements every other day before breakfast, for a total of three supplements per week. For the TABATA with RJ group, two consecutive days of RJ consumption were prescribed on exercise days. Additionally, participants were asked to refrain from taking any other supplements or antioxidants during the training period and to submit the diet checklist provided to them at each training session.

#### 3.6. RNA Extraction and Quantitative Real-time PCR

Total RNA, including LncRNA-H19 and NLRP3 coding RNA, was extracted using a commercial kit (Yekta Tajhiz, Iran) in accordance with the manufacturer's instructions. The concentration and purity of the extracted RNA were determined using spectrophotometry and agarose gel electrophoresis (NanoDrop Iranian, Iran, model NDNM96). Reverse transcription was performed using one microgram of total RNA per sample, an RNA inhibitor, an oligo dT primer, reverse transcriptase (M-MLV RT), and deoxyribonucleotides (YTA, Iran). The complementary DNA (cDNA) was synthesized using a cDNA synthesis kit (Yekta Tajhiz, Iran). Real-time PCR was employed to detect mRNA levels of H19 and NLRP3 (Rotor Gene 6000; Corbett Research, Australia). Specific forward and reverse primers were designed using Primer Premier 5 software for use in QRT-PCR (Table 3). The housekeeping gene  $\beta$ -actin was also measured to normalize the

**Table 2.** High- and Low-Volume High-Intensity Interval Training (TABATA) Exercise Programs Over 8 Weeks

TABATA HIIT Program	Session/Week, Number	Work/Rest, S	1 - 2 Weeks	3 - 4 Weeks	5 - 6 Weeks	7 - 8 Weeks
HV	2	2/1 (20/10)	(2 × 4/1 × 1) <sup>a</sup>	3 × 4/2 × 1	4 × 4/3 × 1	4 × 4/3 × 1
LV	2	1/1 (20/20)	(2 × 5/1 × 1) <sup>b</sup>	3 × 5/2 × 1	4 × 5/3 × 1	4 × 5/3 × 1
RPE (Borg scale 10)	-	7	7-8	7-8	8-9	
S&C exercises	-	S	S	S&C	S&C	

Abbreviations: S, simple; C, complex; HV, high-volume; LV, low-volume.

<sup>a</sup> Example for HV = (2 × 4/1 × 1) = 2 sets × 4 min (8 sequences with a ratio of 20/10 sec) / 1 × 1 min (rest between sets).

<sup>b</sup> Example for LV = (2 × 5/1 × 1) = 2 sets × 5 min (8 sequences with a ratio of 20/20 sec) / 1 × 1 min (rest between sets).

**Table 3.** Characteristics of Forward and Reverse Primers Used in RT-PCR

Genes	Primers	Sequence	Access Number	Base Pair
B-actin	Forward	CGGAAATCGTGCCTGAC	NM_001101.5	109
	Reverse	GCTCGTAGCTCTTCCAGGG		
NLRP3	Forward	GAGCCTCAACAAACGCTACAC	NM_183395.3	151
	Reverse	ATCGGGTCAAACAGCAACT		
lncRNA-H19	Forward	GGAATCGGCTCTGGAAGGT	NR_003491.3	174
	Reverse	TCAGCTCTGGGATGATGGT		

relative expression of each target gene. The  $2^{-\Delta\Delta CT}$  method was used to quantify gene expression (18).

### 3.7. Data Analysis

All analyses were conducted using the statistical package for the social sciences (SPSS) (version 26.0 for Windows, IBM, Armonk, NY, USA). The results of the Shapiro-Wilk test indicated that the data followed a normal distribution. Differences between and within groups for each gene under investigation were assessed using a two-way repeated measures analysis of variance. Additionally, when significant changes were observed, the Bonferroni post hoc test was used to determine differences between groups. Statistical significance was set at  $P < 0.05$ .

## 4. Results

### 4.1. Acute Effects of the Maximal Exercise Protocol (Bruce Protocol)

NLRP3 gene expression during the maximal exercise protocol (Bruce test), before and after the eight-week TABATA HIIT and RJ supplementation interventions, showed an insignificant decrease in the HV group ( $P = 0.271$  and  $P = 0.236$ , respectively), LV group ( $P = 0.355$  and  $P = 0.207$ , respectively), and RJ group ( $P = 0.312$  and  $P = 0.401$ , respectively). In the HV + RJ group ( $P = 0.602$  and  $P = 0.281$ , respectively) and LV + RJ group ( $P = 0.649$  and  $P =$

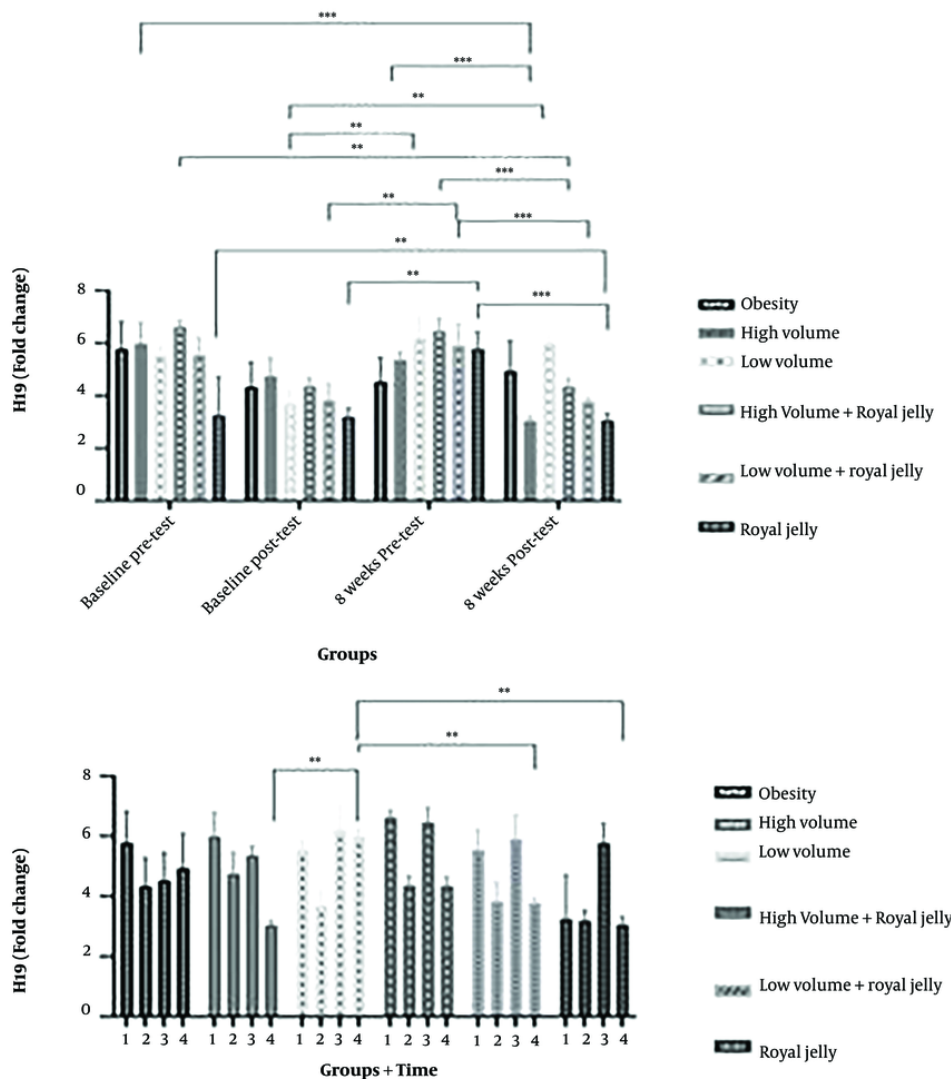
$0.285$ , respectively), there was an insignificant increase. Meanwhile, the changes in NLRP3 gene expression before and after the 8-week interventions did not show any significant difference between the groups studied.

H19 gene expression during the maximal exercise protocol showed a significant reduction in the HV-TABATA HIIT intervention groups, which was more pronounced than in the LV groups before and after 8 weeks: HV group ( $P = 0.296$  and  $P = 0.004$ , respectively), LV group ( $P = 0.125$  and  $P = 0.764$ , respectively), RJ group ( $P = 0.117$  and  $P = 0.001$ , respectively), HV + RJ group ( $P = 0.066$  and  $P = 0.007$ , respectively), and LV + RJ group ( $P = 0.153$  and  $P = 0.002$ , respectively). On the other hand, the maximal exercise protocol after 8 weeks showed a significant difference in H19 gene expression between the HV-HIIT and LV-HIIT groups ( $P = 0.010$ ) (Figures 1 and 2).

Regarding NLRP3 gene expression, the analysis before and after the acute phase (Bruce test) showed an effect size of 0.54 in the LV group. For the H19 gene, the effect sizes were as follows: HV group (0.779), LV group (0.888), HV + RJ group (0.992), and LV + RJ group (0.1009), indicating very strong effect sizes according to Cohen's criteria.

### 4.2. Chronic Effect of TABATA-High-Intensity Interval Training Exercise and Royal Jelly Supplement Interventions

Eight weeks of non-pharmacological interventions involving exercise training and supplementation did

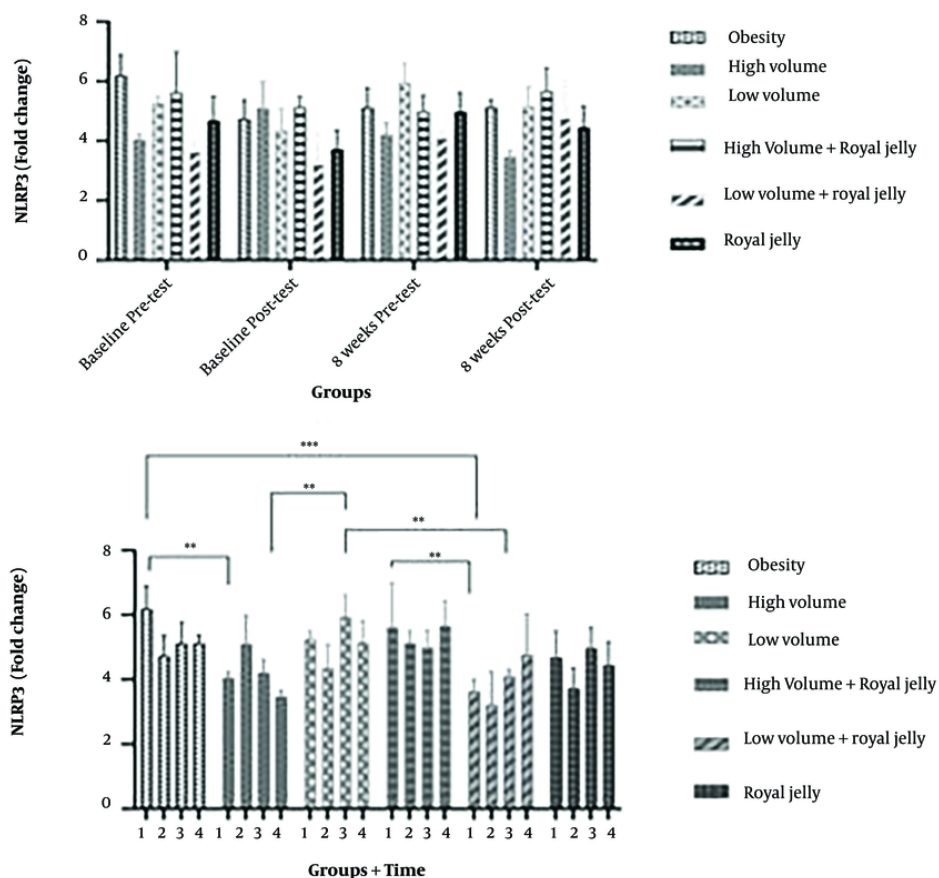


**Figure 1.** Changes in H19 gene expression in the various groups after 8 weeks of high-volume-high-intensity interval training and low-volume-high-intensity interval training exercises, with and without royal jelly interventions. Abbreviations: Baseline pretest, (Before 8 weeks of HIIT); baseline posttest (After 8 weeks of HIIT); HIIT (high-intensity interval training); OBC (obesity control); HV (high-volume HIIT); LV (low-volume HIIT); HV + RJ (high-volume with royal jelly); LV + RJ (low-volume with royal jelly); RJ (royal jelly). 1 (before 8 weeks of HIIT pretest); 2 (before 8 weeks of HIIT posttest); 3 (after 8 weeks of HIIT pretest); 4 (after 8 weeks of HIIT posttest).

not show any significant changes in the resting levels of NLRP3 and H19 genes. However, following 8 weeks of training, a significant decrease in NLRP3 expression and a significant increase in H19 expression were detected in the HV group compared to the LV group, with P-values of 0.05 and 0.010, respectively. Similarly, a significant improvement in resting H19 levels was observed between the RJ and LV groups ( $P = 0.011$ ) and between the LV + RJ and LV groups ( $P = 0.44$ ). Moreover, a

significant reduction in resting NLRP3 expression was found between the RJ+LV and LV groups ( $P = 0.038$ ).

Overall, despite an increase in activity tolerance during the maximal exercise protocol in the intervention groups, the gene expression responses of NLRP3 and H19 after 8 weeks of training with different volumes—HV compared to LV—or using RJ supplementation, were not inhibited (Figures 1 and 2).



**Figure 2.** Changes in NLRP3 gene expression in the various groups after 8 weeks of HV-HIIT and LV-HIIT exercises, with and without Royal Jelly interventions. Abbreviations: Baseline (before 8 weeks of HIIT); HIIT, (high-intensity interval training); OBC (obesity control); HV (high-volume HIIT); LV (low-volume HIIT); HV + RJ (high-volume with royal jelly); LV + RJ (low-volume with royal jelly); RJ (royal jelly). 1 (before 8 weeks of HIIT pretest); 2 (before 8 weeks of HIIT posttest); 3 (after 8 weeks of HIIT pretest); 4 (after 8 weeks of HIIT posttest).

For NLRP3 gene expression, the effect size after chronic exercise training and RJ supplementation was strong in the HV group (0.84), LV group (0.51), and HV + RJ group (0.99). Similarly, for the H19 gene, the effect sizes were strong in the HV group (1.01), HV + RJ group (0.99), LV + RJ group (0.98), and RJ group (0.94), according to Cohen's criteria.

## 5. Discussion

The present study was designed to investigate whether HV or LV HIIT exercises, with and without RJ supplementation, could affect the inflammasome NLRP3 and lncRNA-H19 in obese men aged 40 to 60 years. According to the findings, before the 8-week training period, the expression of NLRP3 and H19 increased due to the maximal exercise protocol on the treadmill. In

contrast, after 8 weeks, the maximal exercise protocol caused a significant difference in H19 and NLRP3 gene expression across the different groups, with this effect being more pronounced in the HV group than in the LV group, especially in those receiving RJ supplementation. In other words, when the same exercise was performed after 8 weeks of regular HIIT exercise and RJ supplementation interventions, activation of the inflammasome was partly corrected due to the significantly decreased NLRP3 expression.

While resting values of H19 gene expression did not show any significant improvement after 8 weeks of HIIT exercise, the resting values of NLRP3 exhibited a significant decrease between the LV and LV + RJ groups compared to the HV group. Hence, our findings indicated that the expression of NLRP3 decreased and

H19 increased following 8 weeks of LV-HIIT and/or HV + RJ-HIIT exercise. This demonstrates that chronic LV-HIIT and/or HV-HIIT exercise with RJ supplementation exerted opposite effects on NLRP3 and H19 expression.

The aforementioned outcome is consistent with other research that demonstrated the gene expression of inflammasomes and the protein expression of inflammatory cytokines, including C-reactive protein (CRP), induced by high-intensity aerobic exercise (19). Prior research suggests that exercise may be useful in reducing the activation of inflammasomes; however, the significance of exercise intensity is still unclear. Therefore, further research is necessary to address the knowledge gaps regarding the precise effects of various exercise types on inflammation. It appears that acute HIIT stimulates and triggers inflammatory pathways and the inflammasome. Conversely, several earlier studies have shown that the duration and intensity of exercise regimens influence the anti-inflammatory and antioxidant benefits of chronic exercise on CV fitness (20). However, it remains unclear how NLRP3 and H19 gene activation are suppressed or upregulated by chronic exercise, respectively. The involvement of the cellular autophagy inflammasome in chronic HIIT may be related to this process. Autophagy can be regulated by components of the inflammasome, which may increase the upregulation of the inflammasome and elevate free radical production. Likewise, regular moderate-intensity training has a beneficial effect on increasing  $VO_{2max}$  and downregulating inflammatory cytokines. Research has demonstrated that engaging in moderate physical activity (60 - 80% of  $VO_{2max}$ ) improves redox balance and positively impacts the function of various tissues. On the other hand, intense exercise sessions may cause oxidative stress and an inflammatory response, among other negative effects. It has been shown that, compared to sedentary control groups, four weeks of resistance training reduced the expression of the NLRP3 gene and blood concentrations of IL-1 $\beta$  and IL-18 (21). Furthermore, RJ is used in the treatment of some diseases due to its anti-inflammatory, antioxidant, and anti-apoptotic properties, as well as its role in improving metabolism. Studies examining the effect of RJ consumption with exercise training have shown that the interaction of both interventions improves motor balance and cognitive function (22).

Even though regular physical activity has many advantages, the molecular mechanisms underlying these effects are still not fully understood. Recent research indicates that members of the NLR family are involved in the assembly of the inflammasome, a molecular platform that helps activate pro-

inflammatory cytokines in response to cellular infection or stress (20). The present study demonstrated a significant reduction in the resting levels of NLRP3, an inflammation-related gene factor, after 8 weeks of HIIT in the HV group compared to the LV group. Furthermore, our study showed a reduction in resting NLRP3 levels and inflammation-related gene factors in the LV + RJ mixed group compared to the LV group, which is consistent with the findings of Petelin et al., who reported that RJ's anti-inflammatory and antioxidant effects significantly reduced the inflammatory marker CRP (12).

In line with the present study's findings, other studies have demonstrated that lncRNAs are differentially expressed after various types of exercise, including HIIT, resistance training, endurance training, and combined training. Long non-coding RNAs expression has been shown to be exercise-specific, with few lncRNAs commonly expressed across different exercise modes (23). Javaid et al. showed that 8 weeks of running on a treadmill, 5 days a week, starting at 12 m/min for 20 min/day in the first week and progressing to 20 m/min for 50 min/day in the last week, had an anti-inflammatory effect and suppressed the inflammatory NLRP3 in adipose tissue (24). Similarly, Niksarasht et al. found that 8 weeks of LV HIIT training had greater anti-inflammatory effects, as indicated by the reduction in H19 and NLRP3 gene expression (25).

On the other hand, Kheirdeh et al. showed that aerobic training and RJ, when administered separately, were effective in improving some inflammatory and regulatory markers, as well as depression and anxiety indices. However, the combination of both had even greater effects (22). Additionally, Yazarlou et al. demonstrated that vitamins play a wide array of biological roles, acting as coenzymes, antioxidants, and hormones, and regulating cellular coagulation and proliferation. Evidence suggests that vitamins and lncRNAs are interconnected through several regulatory axes (26).

Likewise, many studies have demonstrated a close connection between long non-coding RNA H19 and inflammatory genes (27). A growing body of research has shown that overexpression of H19 in hyperglycemia causes an increase in XBP1 expression through miR-93 sponging, leading to a significant decrease in inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 (28). Similarly, 8 weeks of a high-fat diet (HFD) also suppressed H19 levels (29). This implies that circulating H19 may serve as an additional biomarker for type 2 diabetes. However, elevated plasma H19 levels do not always have harmful effects in promoting diabetes (30).



According to the findings of the present study, the HV + RJ-HIIT group showed a significant increase in H19 gene expression compared to other groups. Similar findings have been reported in other studies, indicating that HIIT can influence the quality of adipose tissue and, consequently, the pro- and anti-inflammatory markers secreted by adipokines, leading to favorable metabolic changes in adipose tissue (31).

As far as we know, no studies have yet examined the effects of HV and LV TABATA-HIIT, with and without RJ, on inflammasome NLRP3 and lncRNA-H19 gene expressions in middle-aged men. Accumulating evidence suggests that the use of RJ in conjunction with physical activity can help control inflammation. Royal jelly possesses a wide range of functional properties, including antibacterial, anti-inflammatory, vasodilative, hypotensive, disinfectant, antioxidant, antihypercholesterolemic, antitumor, and estrogenic activities (32). Our results indicated that eight weeks of LV and/or HV + RJ-HIIT interventions led to a decrease in resting NLRP3 gene expression and resting H19 gene expression in healthy men. These findings suggest that chronic RJ supplementation combined with HV-HIIT exercise exerts anti-inflammatory effects.

### 5.1. Limitations

It is important to recognize that the design and interpretation of the current experiment may have some potential limitations. First, the data used in this investigation were obtained from a study of healthy, non-smoking men. Whether these findings can be generalized to females and older populations with chronic obstructive pulmonary disease, cardiac and/or autonomic abnormalities (e.g., diabetic and hypertensive patients) should be further investigated. Considering that smoking can alter inflammasome signaling, this is particularly crucial. Another limitation is the small sample size in our study, primarily due to the prevalence of the COVID-19 pandemic among the age group considered and concerns about the risk of disease transmission.

### 5.2. Conclusions

This was the first study to examine the efficacy of non-pharmacological strategies on coding and non-coding lncRNA genes in obese males. The results of the current human study suggest that chronic TABATA-HIIT exercise combined with the natural supplement RJ can modulate the inflammasome, resulting in downregulation of NLRP3 and upregulation of lncRNA-H19. Although both LV and HV exercises are effective in attenuating inflammatory responses induced by acute

stress, HV exercise combined with RJ intervention appears to offer greater benefits. Further studies are recommended to determine which exercise intensities and antioxidant and anti-inflammatory herbal supplements are more effective in improving the responses of other coding and non-coding lncRNA genes involved in the CV system. Additionally, a comparative study involving both men and women, with a larger sample size and statistical power, is suggested to evaluate differences between healthy individuals and those with chronic diseases (e.g., diabetes, hypertension).

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### Footnotes

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**Data Availability:** The dataset presented in the study is available upon request from the corresponding author during submission or after publication.

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