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Experimental Research Article

Aerobic training and royal jelly enhance thermogenesis genes in the visceral adipose tissue of high-fat diet-induced obese rats

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ABSTRACT

Introduction: The crucial role of adipose tissue (AT) in energy balance has sparked significant interest in researching this tissue as a potential target for obesity treatment. Exercise and dietary interventions are promising strategies for addressing obesity. This study aimed to examine the impact of aerobic training and royal jelly on the expression of thermogenesis-related genes in the visceral adipose tissue (VAT) of obese rats.

Methods: Rats (n=45) were divided into five groups: normal diet (ND), high-fat diet (HFD), high-fat diet-training (HFDT), high-fat diet-royal jelly (HFDRJ), and high-fat diet-training-royal jelly (HFDTRJ). Royal jelly treatment was administered at a dosage of 100 mg/kg body weight. The training was conducted at an intensity of 50-60% VO2max, five days a week for eight weeks. Thermogenesis gene expression was evaluated by the real-time PCR method.

Results: Induction of an HFD significantly reduced the expression of UCP-1, PRDM16, and CREB-1 compared to the normal diet (ND) group (p=0.001). Aerobic training and RJ significantly increased the levels of UCP-1, PRDM16 and CREB-1 in the VAT of HFD rats (p=0.0001). The combined intervention of aerobic training with RJ had no significant effect on the levels of UCP-1, PRDM16 and CREB-1 in the VAT of HFD rats.

Conclusion: It appears that aerobic training and RJ are effective methods for positively regulating the gene expression related to thermogenesis in AT, which may mitigate obesity induced by a high-fat diet.

Keywords:
Exercise
Obesity
Royal jelly
UCP-1
PRDM16
CREB-1

Introduction

Today, obesity and its accompanying epidemic of co-morbidities have become global problems. The World Health Organization (WHO) reports that obesity has nearly tripled worldwide since 1975, with more than 1.9 billion adults being overweight and over 650 million being obese in 2016. Currently, most of the world's population lives in countries where overweight and obesity cause more deaths than malnutrition (underweight), making adipose tissue (AT) a significant challenge to

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chronic disease prevention and overall health globally. This global epidemic can be attributed to advancing economies, the adoption of mechanized transport, urbanization, commercial growth, industrialization, a progressively more sedentary lifestyle, and a nutritional transition to processed foods and high-calorie diets over the last 30 years (Hruby and Hu 2015). Obesity refers to the accumulation of excess mass in white adipose tissue (WAT). It is a significant contributing factor to various metabolic disorders, including dyslipidemia, type 2 diabetes, insulin resistance, and hypertension (Kang and Park 2012). Unlike WAT, which serves as the primary energy reservoir, brown adipose tissue (BAT) plays a crucial role in compensatory thermogenesis. The thermogenic capability of brown adipose cells is largely reliant on the elevated expression of Uncoupling Protein Type 1 (UCP-1) and a high density of mitochondria (Crichton et al., 2017). Research in rats has demonstrated that thermogenesis in brown fat can effectively clear lipids and glucose from the bloodstream, thereby mitigating metabolic diseases. Conversely, the genetic knockdown of brown fat or UCP-1 leads to obesity (Bartelt et al., 2011). Strategies aimed at enhancing BAT, especially through the activation of thermogenesis via UCP-1 in the absence of physiological triggers, could potentially offer therapeutic approaches to address obesity and its associated diseases (Harms and Seale 2013). Studies indicate that PR domain containing 16 (PRDM16) and cAMP Response Element-Binding Protein (CREB-1) are the primary UCP1 gene regulators, ultimately aiding in the activation of brown fat or the regeneration of white fat (Inagaki et al., 2016; Whittle et al., 2012). The PRDM16 protein functions as a crucial activator of the thermogenic gene program in brown and beige fat. When expressed in myoblasts or preadipocytes, PRDM16 suppresses existing gene programs (i.e., myogenic genes or genes associated with white fat) and activates a brown fat-specific gene program, which includes UCP-1, Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α), along with numerous mitochondrial genes (Kajimura et al., 2008). CREB-1 is activated in adipocytes in conditions of obesity, which is also influenced by insulin resistance, resulting in the down-regulation of GLUT4 through the expression of the transcriptional repressor ATF3 (Ghorbani 2015). The CREB protein significantly contributes to glucose homeostasis and metabolism; phosphorylation of CREB in AT cells enhances their activity in response to food, especially in contexts of excessive AT and obesity (Alptarejos and Montminy 2011). Besides preventing obesity by promoting a healthy lifestyle through diet and exercise, one of the best ways for modern-day physicians and scientists to combat the global menace of obesity is to better understand AT. Identifying foods that can encourage the browning of AT presents a potentially effective strategy for addressing obesity. Royal jelly (RJ) is a sticky, jelly-like substance secreted by the hypopharyngeal glands and lymph nodes in the heads of nurse bees (Sabatini et al., 2009). Modern spectroscopic analyses have identified approximately 185 organic compounds within RJ (Najafi et al., 2014). The phenols in RJ exhibit various activities, including antioxidant, antimicrobial, antiviral, antifungal, wound healing, and cardioprotective effects (Zamani et al., 2012). Additionally, RJ has been shown to alleviate diet-induced hyperglycemia and hepatic steatosis by promoting metabolic thermogenesis in AT and reducing insulin resistance (HOMA-IR) in rats (Yoneshiro et al., 2018). Initial studies have suggested that RJ promotes thermogenesis and modulates adipocyte differentiation by influencing transcription factors such as C/EBPα, PPARγ, and SREBP-1c (Mesri Alamdari et al., 2020). Conversely, research indicates that AT can adapt similarly to aerobic exercise as skeletal muscles do (Vidal and Stanford 2020). In obese samples, increased expression of UCP-1 (Mostafavian et al., 2020; Shirkhani et al., 2019) and the PRDM16 gene (Shabani et al., 2018) has been reported following exercise. However, the study conducted by Daneshyar et al. examined the effects of long-term high-fat feeding combined with regular aerobic exercise on the expression of UCP1 genes in rats, revealing that aerobic exercise had no significant impact on UCP1 gene expression (Daneshyar et al., 2021). Additionally, Xu et al. found that continuous endurance exercise did not lead to an increase in PRDM16 gene expression in the epididymal WAT of rats (Xu et al., 2011). Moreover, Karimi et al.'s research demonstrated that CREB protein levels remained unchanged in the subcutaneous fat tissue of obese rats with type 2 diabetes following high-intensity interval training (Karimi et al., 2020). Obesity is currently impacting many individuals worldwide. It is a central factor in the leading causes of illness and death, with obesity-related healthcare expenses costing countries billions of pounds each year. Consequently,

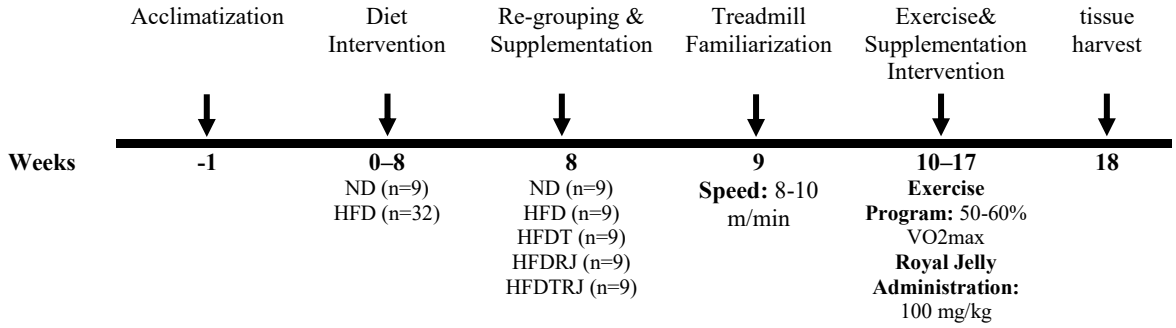

FIGURE 1. Experimental timeline: After a one-week adjustment, Wistar rats were divided into two groups: a control group on a normal diet (n=9, ND) and a high-fat diet group (n=32, HFD). The ND group received a standard diet for eight weeks, while the HFD group was fed a diet comprising 17% protein, 43% carbohydrate, and 40% fat. After eight weeks, all rats were categorized into five groups: ND (n=9), HFD (n=9), high-fat exercise (HFDT) (n=9), high-fat royal jelly (HFDRJ) (n=9), and high-fat sport royal jelly (HFDTRJ) (n=9). Prior to exercise, the mice were acclimated to a treadmill at 8-10 m/min for five sessions over one week. Training was conducted at 50-60% VO2max intensity, five days a week for eight weeks. The HFDRJ and HFDTRJ groups received 100 mg of royal jelly per kg of body weight.

addressing these public health crises necessitates new strategies grounded in a more profound understanding of the tissues and pathways involved in energy balance. RJ has positive effects on energy metabolism and body composition. AT presents a significant target for obesity treatment, as it enhances energy expenditure through non-shivering thermogenesis. The influence of RJ combined with aerobic exercise on the adipose thermogenic genes is not well understood. The researcher hypothesises that the combination of exercise and RJ may have a greater effect on white adipose tissue through its synergy and provide beneficial results for obesity through a change in the phenotype of white adipose tissue to brown. Thus, this study aims to explore the effects of exercise and RJ on the expression of thermogenic genes in the visceral adipose tissue (VAT) of obese rats.

Material and Methods

In this experimental study, 45 male Wistar rats (8 weeks old, weighing 187.51 ± 9.37 g) were maintained under standard conditions (temperature 22 ± 1.4°C, humidity 50%, light-dark cycle 12:12). Water was available ad libitum from a 500 ml bottle. This research received approval from the ethics committee at the *** University, ***, under the reference number ***. Ethical principles for working with laboratory animals were adhered to, including the availability of water and food, appropriate housing conditions, and the absence of coercion during training. After a one-week acclimatization period to the new environment, the rats were divided into two groups: a control group on a normal diet (n=8, ND) and a group on a high-fat diet (n=32, HFD). ND group rats were given a standard diet (23% protein, 65% carbohydrates, and 12% fat) for eight weeks, while the rats in the HFD group were fed an HFD comprising 17% protein, 43% carbohydrates, and 40% fat. The standard food was prepared by Behparvar Company. Due to the lack of high-fat ready-made food pellets, this food was prepared according to the recommendations of livestock and poultry experts. After eight weeks, all rats were divided into five groups: normal diet (ND) (n=9), high-fat diet (HFD) (n=9), high-fat exercise (HFDT) (n=9), high-fat royal jelly (HFDRJ) (n=9), and high-fat exercise royal jelly (HFDTRJ) (n=9) (Figure 1).

Obesity in the rats was assessed using Lee's index, where a level of 310 indicates obesity. Therefore, rats with values exceeding 310 are classified as obese according to the Lee index (Fathi et al., 2015). Immediately after the obesity induction period, the rats began aerobic training and consumed RJ.

Lee index= [body weight $(g)^{0.33}$/ Naso-anal length (cm)] × 10^3

RJ powder was sourced from Bulk Supplements Co., Ltd (Henderson, USA). The HFDRJ and HFDTRJ groups were administered 100 mg of RJ (per kg of body weight), which was diluted in distilled water and given as a daily gavage (between 9:00–9:30 AM) (Mesri Alamdari et al., 2020).

Before commencing the main training, the rats were familiarized with the treadmill for five minutes at a speed of 8-10 m/min and a zero slope, carried out over one week in five sessions. The aerobic exercise program involved running on a treadmill (Omid Iranian Gostar Equipment Company, Made in Iran, 10 lanes) at a zero

TABLE 1: Training protocol

week	1	2	3	4	5	6	7	8
Intensity (m)	15	16	18	20	21	23	25	25
Time (min)	30	35	40	45	50	55	60	60

TABLE 2: Primer sequence of UCP-1, PRDM16 and CREB-1

Genes	Forward primers	Reverse primers
UCP-1	TTCTTTTCTGCGACTCGGAT	GCCCAATGGTGTTTAGCATC
PRDM16	CCAAAACCGTGTGATAAGGTC	GGGTATTTGGCACATTAACAAC
CREB-1	CTACAATATGCACAGACCACT	GAGGACGCCATAACAACTCCA
β-actin	TCAGGTCATCACTATCGGCAA	TTACGGATGTCAACGTCACAC

per cent incline for eight weeks, β five days a week. In the first week, the rats undertook an incremental aerobic exercise regimen on the treadmill with an intensity of 15 m/min for 30 minutes. Subsequently, the intensity increased from 15 m/min to 25 m/min by the seventh week, and the activity duration extended to 60 minutes (Table 1). According to the referenced source, this exercise intensity was equivalent to 50-60% of oxygen consumption (VO2max) in obese rats (Rocha-Rodrigues et al., 2016). To encourage the rats to run, a sound stimulus (a blow against the wall of the treadmill) was employed. In the initial sessions, a low-voltage electric stimulus was applied alongside the sound stimulus. After conditioning the rats to respond to both stimuli, subsequent sessions were conducted, utilizing only the audio stimulus, in order to address the ethical considerations associated with working with laboratory animals.

All animals were anaesthetized under completely similar conditions, following basic protocols (48 hours after the last training session and 12 to 14 hours of fasting), using a combined intraperitoneal injection of ketamine (60 mg/kg) and xylazine (5 mg/kg). Blood samples were taken directly from the inferior vena cava and centrifuged at 300 RPM for 15 minutes after transfer to EDTA-containing test tubes. Fasting glucose was measured by the enzymatic colorimetric method, and insulin by ELISA method. Homeostasis model assessment (HOMA-IR) was used to estimate insulin resistance index. After separation, the visceral adipose tissue of the epidermal region was placed in tubes containing RNA-later solution to prevent RNA degradation, then transferred to liquid nitrogen and stored in a freezer at -80°C until measurement. To mitigate the effects of day and night variations, sampling commenced at 8:00 and concluded at 11:30. Initially, primer design was conducted, after which total RNA was extracted from the tissues and converted into cDNA. Subsequently, the cDNA was amplified via PCR and analyzed for the expression of the specified genes. Table 2 illustrates the primer sequence.

After confirming that the data followed a normal distribution, an independent t-test, two-way analysis of variance, and Tukey's post hoc test were used for statistical analysis at a significance level of $p \leq 0.05$. The statistical software program we used for data analysis was IBM SPSS, version 26.

Results

The average weights of the groups before, during, and after the induction of obesity are presented in Table 3. Additionally, the levels of glucose, insulin, and HOMA-IR in the various research groups are listed in Table 4. The results of the t-test showed that in the first week of obesity induction, there was no significant difference in the rate of weight change between the ND and HFD groups (p=0.836). However, a significant difference was observed in the second (p=0.003), fourth (p=0.001), sixth (p=0.0001), and eighth (p=0.0001) weeks of obesity induction between the ND and HFD groups.

HFD induction caused a significant increase in the changes of HOMA-IR (p=0.0001), glucose (p=0.0001), and insulin (p=0.0001) compared to the ND group. Also, HFD induction caused a significant decrease in the changes of UCP-1 (p=0.0001), PRDM16 (p=0.0001) and CREB-1 (p=0.0001) compared to the ND group (Table 5).

Data analysis using two-way analysis of variance

TABLE 3: The average weight of the groups before and during the obesity induction period

	before induction of obesity			Induction of obesity				
	8-week-old Rat	After adaptation	Grouping	First week	Second week	Fourth week	Sixth week	Eighth week
Age (weeks)	8	9		10	11	14	16	18
Groups	187.51±9.37	200.51±16.26	ND	211.33±19.34	216.33±17.66	245.22±16.51	257.22±22.81	270.11±27.55
			HFD	209.61±22.74	233.56±13.90*	271.89±21.20*	310.58±21.68*	350.83±41.01*

* Difference from the ND group.

Each value represents Mean±SD (n=9/group)

TABLE 4: Levels of glucose, insulin and HOMA-IR in different research groups

	ND	HFD	HFDT	HFDRJ	HFDTRJ
Glucose (mmol/l)	8.06 ± 1.54	14.24 ± 1.93	8.81 ± 1.60	8.98 ± 1.94	7.33 ± 0.91
Insulin (pg/ml)	56.83 ± 9.20	99.83 ± 12.01	76.92 ± 13.46	80.67 ± 11.73	69.85 ± 9.71
HOMA-IR	2.92 ± 0.67	9.07 ± 1.46	4.28 ± 0.89	4.57 ± 0.86	3.27 ± 0.57

TABLE 5: Independent t test results in ND and HFD groups

	p-value	t
Glucose (mmol/l)	0.0001*	-11.434
Insulin (pg/ml)	0.0001*	-8.518
HOMA-IR	0.0001*	-7.497
UCP-1	0.0001*	12.720
PRDM16	0.0001*	11.152
CREB-1	0.0001*	9.495

* Difference from the ND group.
Each value represents Mean±SD (n=9/group)

showed that HOMA-IR, glucose and insulin levels were significantly reduced in the aerobic exercise training (respectively p=0.0001, f2=0.842; p=0.0001, f2=0.666 and p=0.0001, f2=0.611) and RJ (respectively p=0.0001, f^2=0.839; p=0.0001, f2=0.655 and p=0.0001, f2=0.610). Also, the interaction of aerobic training and RJ caused a significant decrease in HOMA-IR (p=0.0001, f^2=0.434) and glucose (p=0.001, f^2=0.232) compared to the effect of each alone. Data analysis using two-way analysis of variance showed that aerobic training (p=0.0001, f^2=0.748) and RJ (p=0.0001, f^2=0.762) caused a significant increase in UCP-1 in the AT of HFD rats. However, the combined intervention of aerobic training with RJ did not have a significant effect on UCP-1 in AT of HFD rats (p=0.148, f^2=0.021). Data analysis using two-way analysis of variance showed that aerobic training (p=0.0001, f^2=0.758) and RJ (p=0.0001, f^2=0.761) caused a significant increase in the expression of PRDM16 in AT of HFD rats. However, the combined intervention of aerobic training with RJ did not have a significant effect on PRDM16 in AT of HFD rats (p=0.285, f^2=0.048). Data analysis using two-way analysis of variance showed that aerobic training (p=0.0001, f^2=0.672) and RJ (p=0.0001, f^2=0.676) caused a significant increase in CREB-1 expression in AT of HFD rats. However, the combined intervention of aerobic training with RJ did not have a significant effect on CREB-1 in AT of HFD rats (p=0.127, f^2=0.017) (Tables 6 and 7).

Discussion

The results indicated that the induction of an HFD led to a significant reduction in the levels of UCP-1, PRDM16, and CREB-1 in VAT. Additionally, aerobic exercise resulted in a notable increase in the values of UCP-1, PRDM16, and CREB-1 in VAT. The increased gene expression of UCP-1 (Mostafavian et al., 2020; Shirkhani et al., 2019), PRDM16 (Shabani et al., 2018), and CREB-1 in VAT following exercise in obese sub-

TABLE 6: Results of Two-Way ANOVA Analysis

		Type III Sum of Squares	df	Mean Square	F	p-value	Partial Eta Squared	Observed Powerc
Glucose	**AT**	212.868	1	212.868	79.815	0.000	0.666	1.000
	RJ	202.932	1	202.932	76.089	0.000	0.655	1.000
	AT×RJ	32.187	1	32.187	12.068	0.001	0.232	0.924
	Error	106.681	40	2.667				
Insulin	**AT**	8062.843	1	8062.843	62.715	0.000	0.611	1.000
	RJ	8035.028	1	8035.028	62.498	0.000	0.610	1.000
	AT×RJ	328.576	1	328.576	2.556	0.118	0.060	0.345
	Error	5142.549	40	128.564				
HOMA-IR	**AT**	190.134	1	190.134	212.857	0.000	0.842	1.000
	RJ	185.522	1	185.522	207.693	0.000	0.839	1.000
	AT×RJ	27.391	1	27.391	30.664	0.000	0.434	1.000
	Error	35.730	40	0.893				
UCP-1	**AT**	280.451	1	280.451	118.559	0.0001	0.748	1.000
	RJ	302.698	1	302.698	127.964	0.0001	0.762	1.000
	AT×RJ	2.035	1	2.035	0.860	0.359	0.021	0.148
	Error	94.620	40	2.365				
PRDM16	**AT**	150.410	1	150.410	125.534	0.0001	0.758	1.000
	RJ	152.483	1	152.483	127.264	0.0001	0.761	1.000
	AT×RJ	2.428	1	2.428	2.027	0.162	0.048	0.285
	Error	47.927	40	1.198				
CREB-1	**AT**	236.109	1	236.109	82.101	0.0001	0.672	1.000
	RJ	240.225	1	240.225	83.532	0.0001	0.676	1.000
	AT×RJ	1.965	1	1.965	0.683	0.413	0.017	0.127
	Error	1513.434	40	1513.434				

jects aligns with our findings. Supporting the current research, Wu et al. demonstrated that long-term exercise in rats enhances UCP-1 levels and promotes the browning of subcutaneous fat tissue, while HFD mitigates these effects (Wu et al., 2014). The primary mechanism responsible for UCP-1 gene expression in brown fat is the beta-adrenergic signaling pathway, which stimulates UCP-1 gene transcription (Wankhade et al., 2016). During aerobic exercise, the secretion of certain hormones that induce the expression of the uncoupling protein gene is elevated, particularly norepinephrine (Kjær 2005), thyroid hormones (Viru and Viru 2001), and the pseudo-hormone Irisin (Roca-Rivada et al., 2013). These hormones promote the transcription of the UCP-1 gene by activating intracellular signaling pathways in WAT, such as PGC-1α, leading to an increase in UCP-1 gene expression over time. UCP1 acts in thermogenesis, the regulation of energy expenditure, and the protection against oxidative stress. All these mechanisms are associated with the pathogenesis of DM2 and obesity. This process contributes to the differentiation of cells into WAT (Bonet et al., 2013). Therefore, it is plausible that part of the effect of exercise on UCP-1 in visceral fat is mediated through the irisin pathway (Kajimura et al., 2015). However, as the levels of these hormones were not measured in this study, this change cannot be definitively linked to the mechanisms of these hormones. So far, several mechanisms involved in PRDM16 gene expression in AT have been identified. One possible mechanism in this relationship is the PPAR-γ protein, which, by binding to another protein called Ebf2, leads to the expression of PRDM16, resulting in the transformation of immature BAT cells into mature ones (Kajimura et al., 2015). In subcutaneous WAT, the binding of PPAR-γ protein to Sirt1 protein also promotes the expression and activation of PRDM16 protein, causing WAT to convert to brown (Roca-Rivada et al., 2013). ZFP423, as a transcriptional regulator, represses the expression of the PRDM16 gene in WAT by inhibiting the transcriptional activity of Ebf2 (Bi et al., 2014). Additionally, it

TABLE 7: Bonferroni test results

	Groups 1	Groups 2	Mean Difference	Std. Error	Sig.[b]
Glucose	**with AT**	**Without AT**	-9.727*	1.089	0.0001
	With RJ	**Without RJ**	-15.749*	1.805	0.0001
Insulin	**with AT**	**Without AT**	-59.862*	7.559	0.0001
	With RJ	**Without RJ**	-99.099	12.535	0.0001
HOMA-IR	**with AT**	**Without AT**	-9.193*	0.630	0.0001
	With RJ	**Without RJ**	-15.058*	1.045	0.0001
UCP-1	**with AT**	**Without AT**	11.164*	1.025	0.0001
	With RJ	**Without RJ**	19.234*	1.700	0.0001
PRDM16	**with AT**	**Without AT**	8.176*	.730	0.0001
	With RJ	**Without RJ**	13.652*	1.210	0.0001
CREB-1	**with AT**	**Without AT**	10.244*	1.131	0.0001
	With RJ	**Without RJ**	17.135*	1.875	0.0001

has been discovered that the Notch signaling pathway is another mechanism that can suppress PRDM16 gene expression in AT (Shao et al., 2016). Both cAMP and cGMP can regulate the phosphorylation of CREB. It has been demonstrated that cAMP stimulates the phosphorylation of CREB at Ser133 and activates it through PKA, whereas cGMP activates the downstream protein cGMP-dependent protein kinase G (PKG), which is a transcription factor that also phosphorylates CREB at Ser133. This dual phosphorylation by PKA and PKG may enhance CREB activity (Heckman et al., 2018). cAMP activates the small GTP-bound protein Rap1 via Epac and triggers the activation of extracellular signal-regulated kinase 1/2 (ERK1/2), which subsequently leads to the phosphorylation of CREB (Grimes et al., 2015). Therefore, it appears that aerobic training may help to regulate CREB in the AT of obese rats by influencing these pathways. Some studies have reported no change in the thermogenic genes within the fat tissue of obese rats (Daneshyar et al., 2021; Karimi et al., 2020; Xu et al., 2011), which contradicts the findings of our study. The discrepancies in the results of these studies can be attributed to factors such as the type of exercise, the specific tissues examined, the subjects involved, and the methods of sampling used.

Various stimuli enhance the function of AT and the thermogenic and fibrogenic markers during obesity, which include pharmaceutical interventions and bioactive compounds. These stimuli can activate beta-adrenergic receptors. Our study's findings indicate that RJ resulted in the upregulation of thermogenic genes in VAT (UCP-1, PRDM16, and CREB-1) and reduced insulin resistance in obese rats. Animal studies have demonstrated that RJ enhances antioxidant effects and ameliorates hyperinsulinemia and insulin resistance by reducing oxidative stress and increasing ATP levels (El-Nekeety et al., 2007). 10H2DA, a fatty acid exclusive to royal jelly, is a key compound for blood glucose regulation (Takikawa et al., 2013). Takikawa et al. discovered that 10H2DA enhances the phosphorylation of non-insulin-dependent AMP kinase (AMPK) in skeletal muscle, increases the GLUT4 to the cell membrane, and thereby elevates intracellular glucose transport (Takikawa et al., 2013). Results from Yanshiro et al. indicate that RJ alleviates diet-induced hyperglycemia and hepatic steatosis by stimulating metabolic thermogenesis in AT and enhances insulin resistance (HOMA-IR). RJ was found to upregulate the expression of the UCP1 gene and protein in AT (Yoneshiro et al., 2018). In a study, Masri et al. investigated the effects of RJ on the activation of BAT and the browning of WAT during caloric restriction in an obese mouse model. The findings indicated that RJ significantly increased the protein expression of UCP1, PRDM16, and CREB1 in both WAT and BAT. The researchers concluded that these outcomes suggest that RJ enhance thermogenesis and WAT browning, contributing to heightened energy expenditure (Fathi et al., 2015). The principal functional compounds in royal jelly, HDEA and HDAA, are integral to its biological activities. They function as agonists of TRPs channels, particularly TRPA1, in gastrointestinal sensory neurons, stimulating thermogenesis in inducible white and classical brown adipocytes through aβ-AR–mediated pathway. Furthermore, they simulate

cold-induced non-shivering thermogenesis (Terada et al., 2011). In the study by Masri et al., the activity of the TRP-SNS-UCP1 pathway is identified as the proposed mechanism behind the thermoregulatory effects of RJ (Fathi et al., 2015). The downstream molecular signaling in the TRP-SNS-UCP1 pathway primarily involves the release of norepinephrine. Tissue stimulation operates mainly through β3-AR, ultimately activating cAMP-dependent PKA. Phosphorylated PKA leads to the phosphorylation of P38MAPK, which sequentially activates the promoters CREB1 and PGC1-α, culminating in UCP1 transcription (Fathi et al., 2015). Therefore, as indicated by the results of our study, RJ may be a dietary option for enhancing the expression of thermogenic genes in AT during obesity. However, the findings of this research suggest that the combination of aerobic exercise and RJ did not prove to be more effective in improving thermogenic fat tissue compared to either exercise or RJ alone. Consequently, the concurrent application of aerobic exercise and RJ does not appear to enhance the effects of these two factors working in tandem to improve the thermogenic indices of AT in obese rats. The lack of significant changes in thermogenic indices in the interaction group may be attributed to the dosage of supplementation and exercise. Since each was effective alone, higher intensity exercise and higher doses of RJ would be required for the difference between the interaction and the effect of each alone to occur. However, there may be other unknown reasons. Therefore, further research in this area is recommended. In the present study, key signaling pathways involved in the onset of brownness and thermogenesis were not assessed in the obese mouse model induced by an HFD, which represents a limitation of this research. It is also recommended to undertake similar investigations by evaluating the thermogenic genes in various regions of AT following aerobic exercise and RJ treatment.

Conclusion

In summary, aerobic exercise and RJ supplementation led to an increased expression of thermogenic genes in the adipose tissue of rats on a high-fat diet. It appears that both aerobic exercise and RJ are effective methods for enhancing the expression of thermogenic genes in adipose tissue affected by a high-fat diet. However, the interactive effect of aerobic exercise and RJ was only significant on HOMA-IR and glucose levels, with no observed effect on other thermogenic indices. Perhaps the dosage of aerobic exercise and RJ needs to be adjusted. Future studies are recommended to explore the interactive role of aerobic exercise and RJ further.

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Conflict of interest

The authors declare that they have no competing interests.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Islamic Azad University, Ayatollah Amoli Branch (No. IR.IAU.M.REC.1400.020).

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