The Effects of Royal Jelly and Tocotrienol Rich Fraction Along with Calorie Restriction Diet on White Fat Browning and Brown Adipocytes Activation in Obese Rats

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Research

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Abstract

Background: Obesity is a public health problem across the world. Development of beige adipocytes in white adipose tissue (WAT) and activation of brown adipose tissue (BAT) can support obesity management. We aimed to investigate the effects of royal jelly (RJ) and tocotrienol-rich fraction (TRF) along with calorie restriction diet (CRD) on the genes involved in beige fat formation and BAT activation.

Methods: Fifty 3-week-old male Wistar rats were fed high-fat diet (HFD) for 17 weeks. When obesity was induced, they were randomly divided into 5 groups (n=10/group): HFD, CRD, RJ+CRD, TRF+CRD, RJ+TRF+CRD for an additional 8 weeks. Finally, body weight was measured. Moreover, WAT and BAT were dissected for assessing the expression of major genes involved in adipose thermogenesis and histological changes evaluation.

Results: At the end of the intervention, weight significantly decreased in RJ and RJ+TRF groups relative to the CRD group (p<0.05). RJ remarkably increased the expression of uncoupling protein 1 (UCP1) by 5.81 and 4.99 times more than CRD alone in WAT and BAT respectively (p<0.001). Expression of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1-α), peroxisome proliferator-activated receptor-α (PPAR-α) and Sirtuin1 (SIRT1) was significantly increased in WAT and BAT of rats receiving RJ and RJ+TRF. Peroxisome proliferator-activated receptor-γ (PPAR-) expression was not noticeably changed in assessed adipose tissues. Brown-like adipocytes in WAT and denser adipocytes in BAT were obvious in RJ and RJ+TRF groups. However, the effect of TRF on studied genes was not noticeable.

Conclusion:

RJ+CRD improved markers of adipose thermogenesis and induced anti-obesity effects more than CRD alone did. Furthermore, RJ remodeled adipose tissue and could be considered as a new therapeutic target.

Introduction

Obesity constitutes one of the serious health problems in the world. Increasing its prevalence at an alarming rate is indisputable and associated with increased all-cause mortality (1). In 2016 more than 1.9 billion adults were overweight (2). Calorie restriction diet (CRD) is known as a common strategy for the treatment of obesity. However, the magnitude of weight loss through CRD is lower than what is expected and weight loss maintenance is a great challenge (3, 4). Some previous studies suggested that poor success in weight loss with CRD may be related to the suppression of uncoupling protein 1 (UCP1) expression in adipose tissues, contributing to energy conservation as an adaptive response (3, 5–7).

Classic brown adipose tissue (BAT) with a high content of mitochondria and high expression of UCP1 is a specialized tissue that has attracted a great deal of attention in obesity management. white adipocytes have a buffering role and containing a single large lipid droplet and UCP1 doesn’t express in WAT naturally (5, 8). Brite (brown-in-white) or beige adipocytes, are cells with more oxidative phenotype similar

to brown adipocytes that are present in white adipose tissue (WAT). Thus beige fat formation via adipose tissue remodeling which described as “browning/ beiging process” could be a therapeutic target in obesity. Browning process results in elevated thermogenesis and negative energy balance (9, 10).

peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1-α) and PR domain containing16 (PRDM16) are two major transcriptional regulators that together with other regulators such as peroxisome proliferator-activated receptors (PPARs) bind to UCP1 promotor and consequently, induce beige adipocytes formation and also trigger BAT activation. Sirtuin1 (SIRT1) has a key role in PGC1-α and PRDM16 activation (8, 9, 11).

Nowadays, regarding weight loss problems via CRD, more options with thermoregulatory properties are increasingly demanded for achieving and maintaining weight loss. A growing body of evidence has documented that functional and nutraceutical food could play roles in adipose tissue remodeling and obesity treatment (12, 13)

Royal jelly (RJ) as an important product of honey bees, is a functional food that provides a wide range of effective ingredients conferring its beneficial health effects (14–17). Meanwhile, a great deal of interest has been directed toward RJ because of its fatty acid composition including trans-10-hydroxy-2-decenoic acid (HDEA) and10-hydroxydecanoic acid (HDAA) (16, 18). Thermogenesis effects of RJ via stimulation of transient receptor potential ankyrin 1 (TRPA1) have been suggested (17). Furthermore, previous researches have demonstrated that the administration of RJ increased PGC-1α mRNA levels in animal models (19, 20). A recent study reported that RJ causes elevation in UCP1 expression and BAT activity in mice (21).

In addition, evidence from previous studies indicates that tocotrienol (T3) and tocotrienol-rich fraction (TRF) which found in plant products such as rice bran and vegetable oils including palm oil and grapeseed oil have significant health-promoting effects (22–24). Since TRF improves the activity of PPARs and acts like PPAR ligands, it is likely to be effective in beige fat formation and BAT activation (25, 26).

To the best of our knowledge, no study has evaluated the effect of RJ and TRF, as a complementary therapy, on thermogenesis and adipose tissue remodeling along with CRD. Given that, this study was conducted to examine whether UCP1 expression in WAT and BAT diminish with weight loss. Furthermore, WAT to BAT remodeling and BAT activation were assessed when RJ and TRF are consumed along with CRD and compared with CRD alone.

Method

Animals

All procedures involving animals and their care were conducted in accordance with National Institutes of Health guide for the care and use of laboratory animals (27). The experimental protocol was reviewed

and approved by the Ethics Committee of Iran University of medical sciences (ethic code: IR.IUMS.FMD.REC 1396.9321324002). Male Wistar rats aged 3 weeks and weight of 58 ± 4 g were obtained from the Pasteur Institute (Tehran, Iran). They were individually housed in stainless steel cages in an air-conditioned room (21–24 °C, 50–60% relative humidity) with a 12/12 h light/dark cycle.

Experimental Design, Diet And Treatment

Our study consists of two phases: i) obesity induction; ii) intervention in obese rats (Fig. 1). The rats were acclimatized for one week after arrival and then randomly assigned to two groups including HFD group fed Semi-purified HFD (n = 50) and normal diet (ND) group which fed standard laboratory chow-diet. We mixed standard chow powder with milk butter (40% w/w) and prepared HFD. The compositions of HFD and ND are shown in Table 1. High-fat pelleted diet was prepared and stored at 4 °C and then used freshly within two days of preparation.

Table 1
Composition of consumed diets

Dietary Composition(g/kg)	ND	HFD	CRD
Carbohydrate	536.2	335.125	335.125
Fiber	42	26.25	26.25
Protein	260.8	163	163
Lipid	40	400	400
Calcium	9.5	5.93	5.93
Phosphorus	6.5	4.06	4.06
Salt	5	3.125	3.125
Moisture	50	31.25	31.25
Ash	50	31.25	31.25
Energy density (kcal/g)	3.6	5.6	5.6
ND: normal diet; HFD: high fat diet; CRD: calorie restriction diet			

Animals had free access to food and water during the obesity induction phase. We weighed animals every week and compared the two groups with each other to assess HFD-induced obesity. The mean weight of rats in the HFD group was elevated significantly relative to the control ND after 17 weeks (443.28 ± 46.62 g vs 396.24 ± 28.79 g; $P < 0.05$). Further, the obesity model was induced in the HFD group. In the second phase of the study, obese rats were randomly divided into five groups (n = 10) matched for body weight for eight weeks including **a)** RJ group; 100 mg/kg/day RJ orally added to CRD **b)** TRF group; 85 mg/kg/day TRF orally added to CRD **c)** RJ + TRF group; both 100 mg/kg/day RJ with 85 mg/kg/day

TRF orally added to CRD **d)** CRD group; CRD with no added RJ and TRF as control for RJ, TRF and RJ + TRF groups and **e)** HFD group; HFD with no added RJ and TRF as control for CRD group.

Administered doses of RJ and TRF were chosen considering previous studies based on no observed adverse effects (24, 28). The composition of CRD was similar to HFD but the calorie of CRD was 30% lower than the amount of calorie in ad libitum intake of HFD (Table 1). The CRD was weighed then determined amount of RJ and TRF were added and then supplemented food was fed to rats. Animals had free access to food in the HFD group. We purchased Lyophilized RJ powder from Bulk Supplements Co, Ltd, (Henderson, USA) with 6% of 10-H2DA. TRF was obtained from ExcelVite Co, Ltd (Perak, Malaysia) and comprise α-tocotrienol (12%), β-tocotrienol (2%), γ-tocotrienol (19.3%) and δ-tocotrienol (5.5%) together with α-tocopherol (11.9%). At the end of the experiment, animals were kept fasting overnight and blood was collected via cardiac puncture from rats anesthetized with xylazine and ketamine.

Two parts of the inguinal WAT and interscapular BAT were dissected, cleaned off their adhering tissues and washed with phosphate-buffered saline (PBS) solution. One part was frozen at − 80 °C in RNAlater stabilization solution (Qiagen, Inc. Germany) for determination of genes expression levels with real-time reverse transcription polymerase chain reaction (RT-PCR), while the other part was fixed in 10% formalin buffered solution for 7 days at room temperature for histological assessment.

Rna Isolation And Quantitative Real-time Pcr

Total RNA was isolated from adipose tissues using Trizol Reagent (Thermo fisher, USA). The quality and quantity of extracted RNA were evaluated spectrophotometrically (NanoDrop One/Onec, Thermo Scientific). Reverse transcription of total RNA to complementary DNA (cDNA) was conducted using the RevertAid First Strand cDNA Synthesis Kit

(Thermo Scientific, USA). The cDNA was prepared from 1 µg of mRNA and was subjected to RT-PCR on a quantitative PCR System. PCR amplification was performed with a fluorescence thermal cycler (Light Cycler system; Roche Diagnostics, Mannheim, Germany) system using SYBR green kit (Takara Bio Inc., Shiga, Japan) and rat specific primer sequences targeting the genes including UCP-1, PGC1-α, PPAR-α, PPAR-γ, SIRT1 and β-actin. The specific forward and reverse primers for the assessed gene were designed using the NCBI Primer Bank and were got from Metabion international AG (Steinkirchen, Germany). The sequences of primers are tabulated in Table 2.

Table 2
List of rat-specific primers used in qRT-PCR

Gene	Forward	Reverse
UCP-1	TTCTTTTCTGCGACTCGGAT	GCCCAATGGTGTTTAGCATC
PPAR-α	GGACTTGAATGACCAGGTTAC	TCAGCATCCCGTCTTTGTTCA
PPAR-	GTTCGCCAAGGTGCTCCAGAA	AAGGCTCATATCTGTCTCCGT
SIRT1	CTCTGAAAGTAAGACCAGTAG	ACATCGCAGTCTCCAAGAAGC
PGC1-α	TACACAACCGCAGTCGCAAC	TCCACACTTAAGGTTCGCTCA
β-actin	TCAGGTCATCACTATCGGCAA	TTACGGATGTCAACGTCACAC

The cDNA was amplified in the three steps: 95 °C (10 min), 95 °C (10 s), 60 °C (10 s), for 45 cycles with 100% ramp rate. Relative expression of the genes of interest was calculated as relative Ct value and normalized to housekeeping gene (β-actin) (29). We analyzed triplicate Ct values for each sample.

2.5. Histological Assay

For histological examination, WAT and BAT that fixed in 10% formalin were dehydrated using different solutions of alcohol and then embedded in paraffin. Paraffin-embedded tissues were sectioned at a thickness of 5 μm cut by microtome and stained with hematoxylin and eosin (H&E). The sections were viewed under the microscope (magnification, X400).

The sections were viewed triplicate by a skilled histologist under the microscope (magnification, 40X). we conducted histomorphometric studies based on our previous work (30). Actually, 100 sections of each sample of WAT and BAT (10 microscopic fields that were selected randomly and 10 lams were provided from each sample) were assessed and the mean percentage of evaluated adipose tissue (BAT, WAT and beige) and connective tissue were considered for each group.

2.6. Statistical Analysis

One-sample Kolmogorov-Smirnov test was used to analyze the normality of data. All Data were expressed as mean ± SEM. Differences between groups were tested using one-way analysis of variance (ANOVA), with Tukey's post hoc test analysis for multiple comparisons.

The results of gene expression were presented as fold changes. All analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc. Chicago, IL, USA, version 21). The Prism software, version 6·0 (GraphPad, CA, USA) was used for drawing figures. A P value < 0.05 was considered statistically significant.

Results

The effect of CRD, RJ and TRF in weight loss

Obese animals fed CRD for eight weeks following 17 weeks HFD had a significant weight loss ($p < 0.001$). The weight loss was 40.70 ± 6.50 g for the CRD group. The rats gained weight about 37.04 ± 5.56 g in the HFD group. In both groups, the rate of weight changes gradually decreased over the eight weeks (Fig. 2A). Addition of RJ, TRF and RJ + TRF to CRD respectively caused 67.21 ± 4.84 g, 44.40 ± 3.35 g and 73.29 ± 4.51 g weight loss relative to starting point of intervention (Fig. 2B). Final weight in RJ and RJ + TRF groups reached statistically significant levels in comparison to the CRD group ($p < 0.05$). However, no significant weight loss was detected in TRF groups relative to the CRD group ($p \geq 0.05$).

The effect of CRD alone and along with RJ and TRF on UCP1 expression

As shown in Fig. 3A, CRD induced about 36% reduction in UCP1 expression in WAT and also decreased UCP1 expression by 14% in BAT as compared with HFD which did not reach statistically significant levels ($p \geq 0.05$). The level of UCP1 mRNA in the RJ group was 5.81 and 4.99 times more than the CRD group in WAT and BAT, which was statistically significant ($p < 0.001$). Dietary TRF resulted in elevation of UCP1 mRNA level in both WAT and BAT when compared with the CRD group, but the changes were not significant. The mRNA level of UCP1 was shown to be significantly higher in the RJ + TRF group as compared to the CRD group in both WAT and BAT ($p < 0.001$) (Fig. 3B).

Assessing the effect of RJ and TRF on some genes that regulate UCP1 expression in adipose tissues

We evaluated the expression of PGC-1α as a key regulator of thermogenesis and expression of UCP1. The mRNA levels of PGC1-α in WAT and BAT of rats in RJ groups increased considerably by 3.65 and 2.75 folds relative to CRD group ($p < 0.001$). However, the changes of PGC1-α expression in the TRF group did not reach significant levels in any of the assessed tissues ($p \geq 0.05$). Furthermore, the expression of PGC1-α increased significantly in the RJ + TRF group to 3.25 and 2.9 fold in both aforementioned tissues when compared with CRD alone ($p < 0.001$) (Fig. 4A).

Interaction between PGC1-α and PPAR- α/ γ form important thermoregulatory complex, involved in UCP1 induction and adipose tissue remodeling. So we assessed the mRNA levels of PPAR-α, PPAR-γ in WAT and BAT.

As shown in Fig. 4B, in comparison with the CRD group, RJ and RJ + TRF groups could enhance the PPAR-α expression considerably in WAT and BAT ($p < 0.001$). TRF could not significantly increase the expression of PPAR-α more than CRD, in WAT and BAT of obese rats ($p \geq 0.05$). RJ and TRF, neither separately nor together in the RJ + TRF group, significantly increased the mRNA levels of PPAR-γ more than CRD did in WAT and BAT ($p \geq 0.05$) (Fig. 4C).

In addition, SIRT1 has a remarkable role in thermogenesis via post-translation modification of transcriptional regulators. Then, we investigated the effect of RJ and TRF on SIRT1 expression. The

mRNA levels of SIRT1 increased dramatically in RJ and RJ + TRF groups in both investigated adipose tissues in comparison with the CRD group ($p < 0.001$). SIRT1 expression, neither in WAT nor in BAT were significantly altered in the TRF group ($p \geq 0.05$) (Fig. 4D).

Histological Results

The histological results of WAT of rats were shown in Fig. 5A. In the HFD and CRD group, there were no changes in favor of WAT remodeling. The histology of WAT in the CRD group showed unilocular white adipocytes with smaller lipid droplets relative to HFD. Remarkably, multilocular beige adipocytes in WAT of rats in the RJ and RJ + TRF groups were obvious. However, the WAT browning in the TRF group was so limited than other intervention groups. When we assessed the BAT (Fig. 5B) in the HFD group, BAT underwent an adverse remodeling in some parts and white adipocytes with large lipid droplets could be distinguished near brown adipocytes. Some white adipocytes close to BAT were seen in the CRD group but at a limited level as compared with HFD. Interscapular BAT in RJ and RJ + TRF groups had a more reddish-brown color appearance, more adipocyte density and multilocular brown adipocytes relative to CRD group. While interscapular BAT in the TRF group has white-reddish color and was less dense.

Discussion

Previous evidence suggested that some problems in weight loss occurring with CRD result from adaptive response for energy saving in calorie restriction condition. So in the current study, we evaluated the effect of RJ and TRF supplementation along with CRD on the weight loss, WAT to BAT remodeling and BAT activation relative to CRD alone in obese rats.

Slowing weight loss phase following fast weight loss and weight recidivism along CRD has been reported in several studies (3, 4, 31). In our investigation, both fast and slow phases of weight loss were observed. When assessing the trend of weight loss, we found that the slope of weight loss dramatically decreased after day 40. Adding RJ and TRF to CRD caused remarkable weight loss in RJ and RJ + TRF, but not in the TRF group. Both RJ and RJ + TRF attenuated reductions in weight loss slope occurring with CRD in days 40 to 60. This observation prompted a closer look at RJ and TRF on thermogenesis via adipose tissue remodeling, UCP1 regulation and related genes expression to elucidate the mechanism underlying this process.

Our finding indicated that eight weeks CRD noticeably decrease the UCP1 expression by 36% and 14% in WAT and BAT of the obese rats as compared with the HFD group but could not reach statistically significant levels. In the current work, RJ supplementation reversed the decrement of UCP1 expression following CRD in the obesity model of rats. To the best of our knowledge, no research has assessed the effect of RJ during CRD on UCP1 expression and beige fat formation. Only one study by Yoneshiro et al. reported BAT activation properties of RJ in obese rats along with HFD (21).

We recently assessed the effect of RJ and TRF on UCP1 overexpression via TRP-SNS-UCP1 pathway (32). Enhancement of β-adrenergic action and 5'-adenosine monophosphate-activated kinase (AMPK) by TRPA1 activators triggers P38MAPK expression and is an important aspect that may favor the UCP1 expression effects of RJ (11, 17, 32). Our previous study revealed that RJ remarkably elevated the level of P38MAPK mRNA in both WAT and BAT of rats along with CRD (32). Since P38MAPK signaling is one of the major pathways involved in adipocytes browning we measured some of P38MAPK target genes expression such as PRDM16 and CREB1 (33). RJ induced the PRDM16 and CREB1 mRNA levels in previous work (32). So in the current study, we evaluated some other target genes for P38MAPK signaling in PGC-1α dependent pathway.

The Expression of PGC-1α was significantly enhanced in the WAT and BAT of RJ and RJ + TRF groups as compared to CRD. Previous works have demonstrated that the administration of RJ increased PGC-1α mRNA levels in the liver and skeletal muscle of obese mice (19, 20). PGC-1α is an important transcription factor that regulate biogenesis of mitochondri and thermogenic reflex by forming complexes with PPAR-α/ PPAR-γ and binding to a PPAR response element in the UCP1 promoter to induce its expression. Notably, PRDM16 also interacts with the aforementioned complex and has been identified to play a pivotal role as a UCP1 expression inducer (9–11). We found out PRDM16 overexpressed following RJ consumption in previous work (32). As pointed above, PPAR-α has been identified to play a pivotal role as a UCP1 expression inducer (8, 34). Assessing the changes of PPAR-α mRNA level showed that RJ dramatically boosted PPAR-α expression in WAT and BAT. Our results are in line with Yoshida et al. study revealing PPAR-α amelioration by RJ supplementation in the liver of obese mice (19). Our results indicated that RJ could not induce PPAR-γ relative to the CRD group. PPAR-γ is a key part of the thermogenesis process and master regulator of lipogenesis (8, 9). It is reasonable that RJ may exert its anti-obesity effects in part through the induction of PPAR-α without any change in the PPAR-γ level.

In addition, SIRT1 is another key gene recognized in beiging process which plays an important role in AMPK/SIRT1/PGC1-α pathway and assessed in this study. In the current work, SIRT1 was overexpressed in WAT and BAT of the RJ group when compared to the CRD group. Post-translation modification is a further critical step that regulates beige fat formation. In this regard, SIRT1-depended deacetylation of PGC1-α and PPARs contributes WAT remodeling. So SIRT1 has a critical role in the induction of genes typical for BAT and leads to PRDM16 and PGC1-α activation, which in turn activates PPARs, and eventually trigger UCP1 expression and thermogenesis enhancement (10, 11) (Fig. 6).

Given that, RJ enhanced gene-related thermogenesis, beige fat formation and BAT activation confirming with histological assessment in the current work.

TRF did not show any differences in thermogenesis and expression of the aforementioned genes in comparison with the CRD. Fang et al. did not find any remarkable effect either on PPARs expression in mice feeding TRF, though the increase in PPARs gene expression and activity was observed *in vivo* part of their study (26). Further, no remarkable changes were reported in another animal model study in PPARs expression after TRF consumption (35). We would probably achieve more remarkable results if we added

T3 instead of TRF. α-tocopherol exert inhibitory effects against the bioavailability of T3 and suppresses its effect; so their possible interaction cannot be ignored (36). However, due to the lack of knowledge about T3 supplementation along with CRD, further research warranted to elucidate the involved mechanisms.

Remarkable improvement in adipose tissue thermogenesis and the expression of assessed thermogenic genes were shown in obese rats treated with RJ + TRF similar to RJ-fed rats. considering the slight effect of TRF, it is therefore likely that the RJ is responsible for the studied changes in the RJ + TRF group. The minor difference between the two aforementioned groups may have resulted from the individual differences in rats in RJ and RJ + TRF groups.

Our data demonstrated RJ improved adipose tissue thermogenesis and remodeling along with CRD and consequently caused more weight loss. However, it is necessary to mention some limitations of recent investigate which are suggested to be considered in future studies. Firstly, evaluation of some other possible dependent-CNS pathway regulating browning/beiging process such as the effect of RJ on endoplasmic reticulum stress reduction in the hypothalamus which can activate CNS signaling and then thermogenic genes in adipose tissue. Secondly, we investigated the effect of γT3 within TRF which may have interacted with other components in TRF. So designing further studies with γT3 is suggested. Finally, we couldn't assess the effect of RJ and T3 in different doses. We suggest more studies to evaluate dose-dependent manner of RJ and T3 in the obesity model.

Conclusion

Generally, in our investigation, we could separate two fast and slow phases of weight loss with CRD. Supplementation with RJ and RJ + TRF attenuated reductions in weight loss slope occurring with CRD. Furthermore, our finding potently revealed the effect of RJ on PGC1-α expression in WAT and BAT of obese rat during CRD. The thermogenic effects of RJ via SIRT1/PGC1-α signaling and forming PGC1-α/PPAR-α/PRDM16 complex were assessed. Based on current findings and regarding our previous data, supporting the effect of RJ on TRP-SNS-UCP1 pathway, we concluded the effect of RJ on the beige fat formation and BAT activation in the rat obesity model. It seems that consuming RJ along with CRD induce some anti-obesity effects more than CRD alone did and could be considered as a new approach to weight management. Studies that examine the effect of RJ and TRF along with other kinds of diet can be extremely helpful in this regard.

Abbreviations

WAT: white adipose tissue; BAT:brown adipose tissue; RJ:royal jelly; TRF:tocotrienol rich fraction; CRD:calorie restriction diet; HFD:high-fat diet; UCP1:uncoupling protein 1; PPAR-α:peroxisome proliferator-activated receptor-α; SIRT1:sirtuin1; PGC1-α:peroxisome proliferator-activated receptor-γ coactivator 1α; PPAR-γ:peroxisome proliferator-activated receptor-γ; PRDM16:PR domain containing16; P38 MAPK:P38 mitogen-activated protein kinase); HDEA; trans-10-hydroxy-2-decenoic acid; HDAA:10-hydroxydecanoic

acid; TRPA:transient receptor potential ankyrin; T3:tocotrienol; PBS:phosphate-buffered saline; RT-PCR:real-time reverse transcription polymerase chain reaction; H&E:hematoxylin and eosin, ANOVA:analysis of variance; SPSS:Statistical Package for the Social Sciences; AMPK:adenosine monophosphate-activated kinase

Declarations

Ethics approval and consent to participate

The experimental protocol was reviewed and approved by the Ethics Committee of Iran University of medical sciences (ethic code: IR.IUMS.FMD.REC 1396.9321324002).

Consent for publication

Not applicable

Availability of data and materials

The datasets were used in current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest.

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Author Contributions

All authors were involved equally in all parts of this study. MR.V, F.sh and A.S supervised the work, designed research and overseeing the study implementation. P.I and N.MA performed experiments, developed the hypothesis, and wrote the manuscript. M.AJ analyzed and interpreted data. N.R and M.A advised in RT-PCR experiments. Sh.A and N.AH assisted in technical experiments and laboratory works. L.R participated in histological examinations. F.F critically read and approved the manuscript. All authors approved the final draft.

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Figures

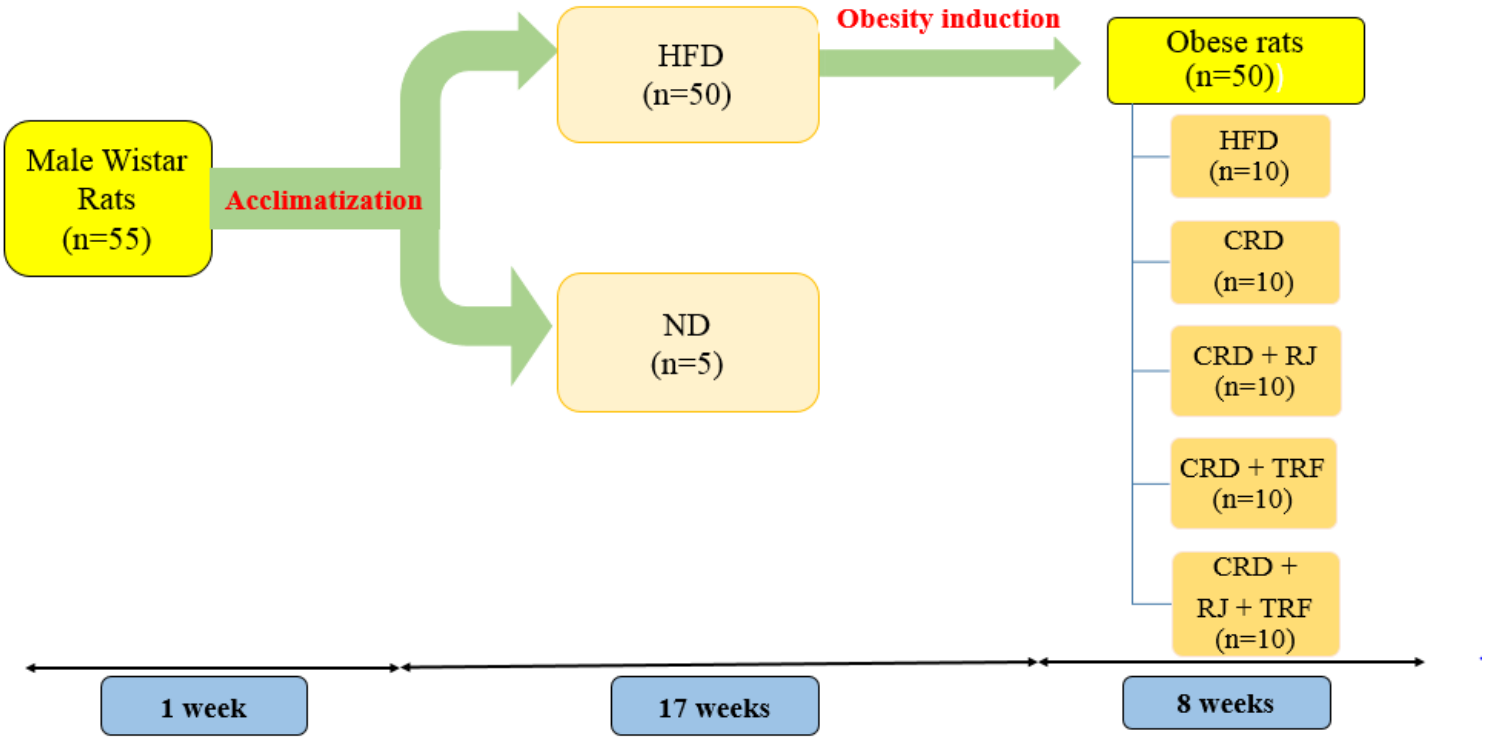

Figure 1

Scheme of study protocol

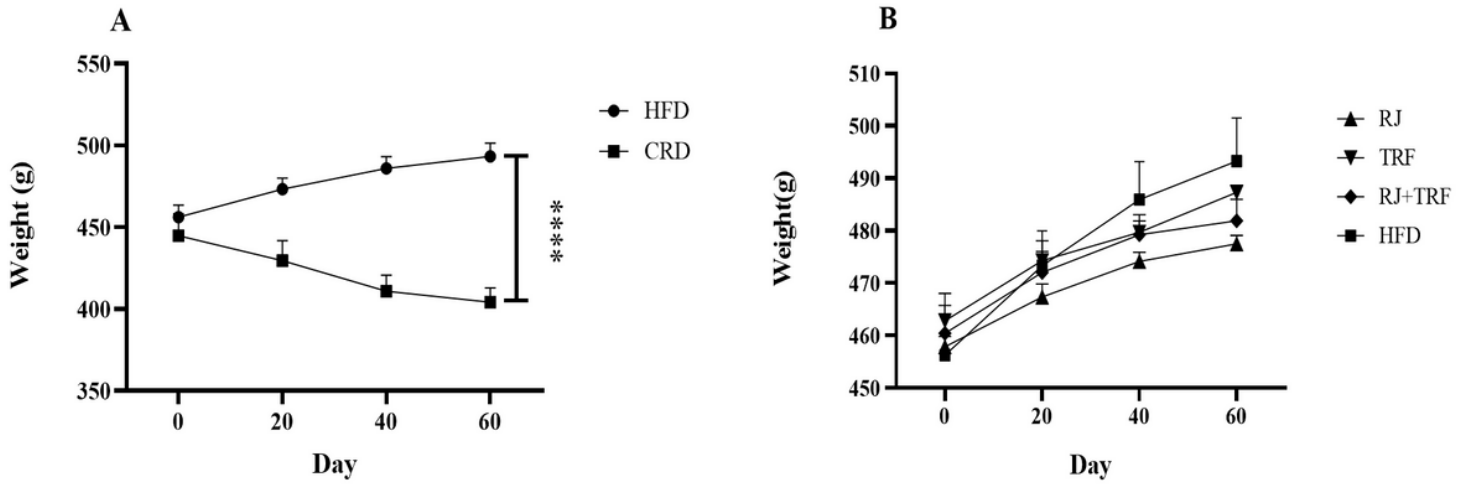

Figure 2

Weight changes during 60 days, (A) between CRD (n=10) and HFD (n=10) ; (B) between the RJ (n=10), TRF (n=10), RJ+TRF (n=10) and CRD groups; Data shown as mean ± SEM; **P < 0.05, ****P <0.001 versus control

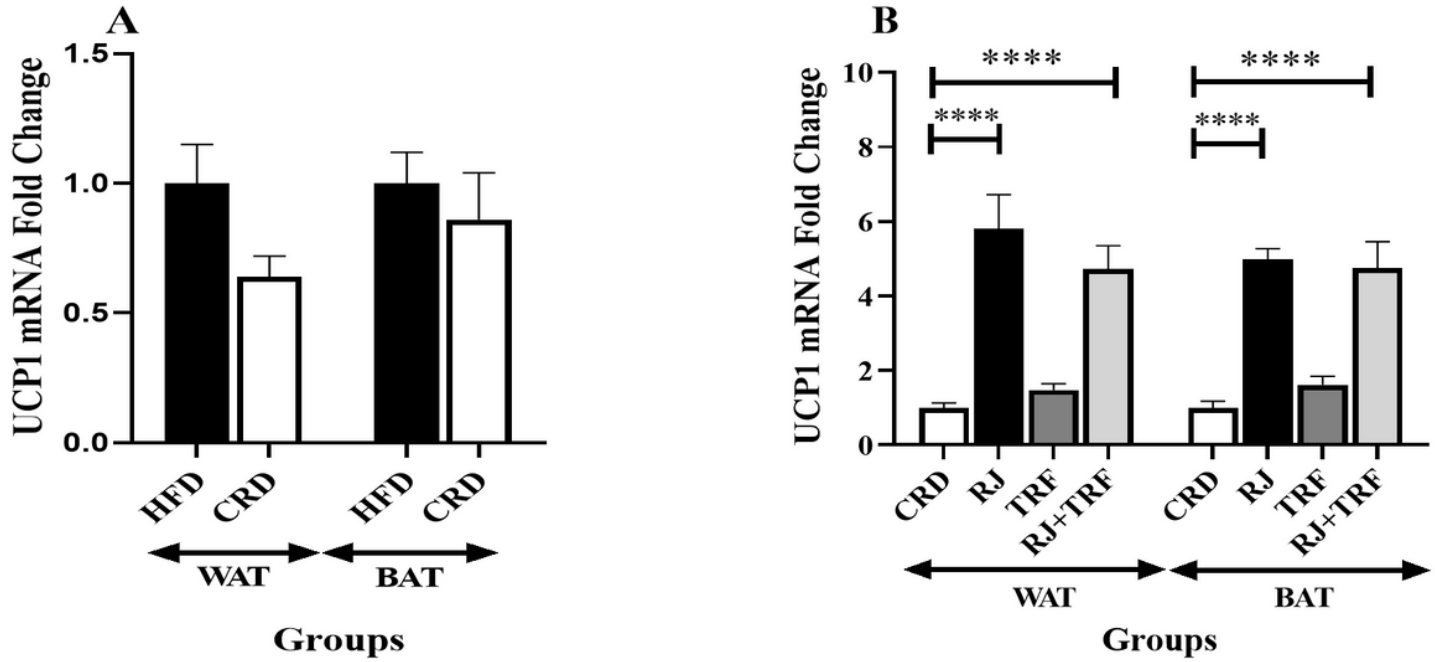

Figure 3

(A) UCP1 mRNA fold change in CRD (n=10) vs HFD (n=10) in WAT and BAT; (B) mRNA fold change in RJ (n=10), TRF (n=10) and RJ+TRF (n=10) in WAT and BAT vs CRD for UCP1. Data shown as mean ± SEM; **P < 0.05, ****P <0.001 versus control

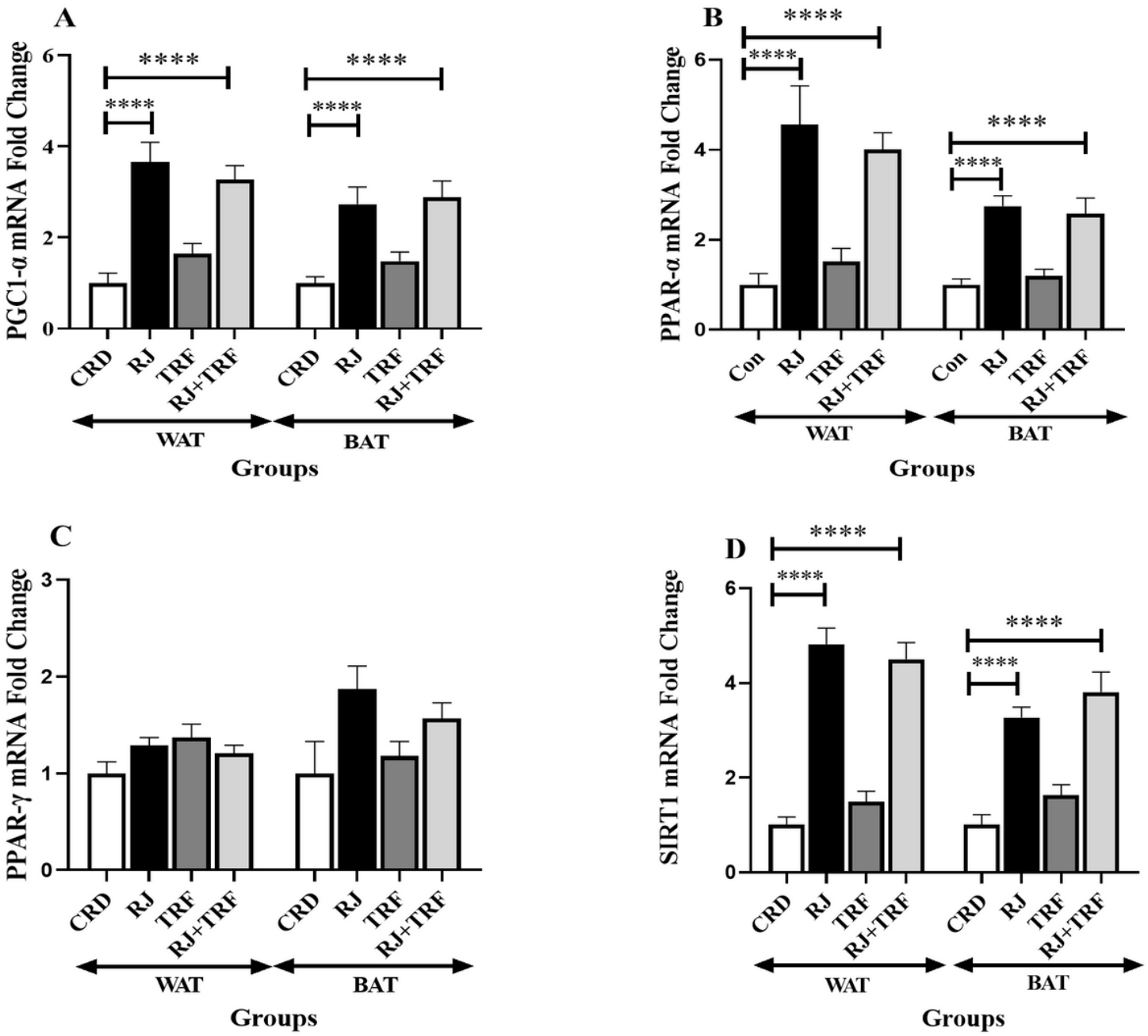

Figure 4

mRNA fold change in RJ (n=10), TRF (n=10) and RJ+TRF (n=10) in WAT and BAT vs CRD for (A) PGC1-α; (B) PPAR-α; (C) PPAR-γ; (D) SIRT1. Data shown as mean ± SEM; **P < 0.05, ****P <0.001 versus control

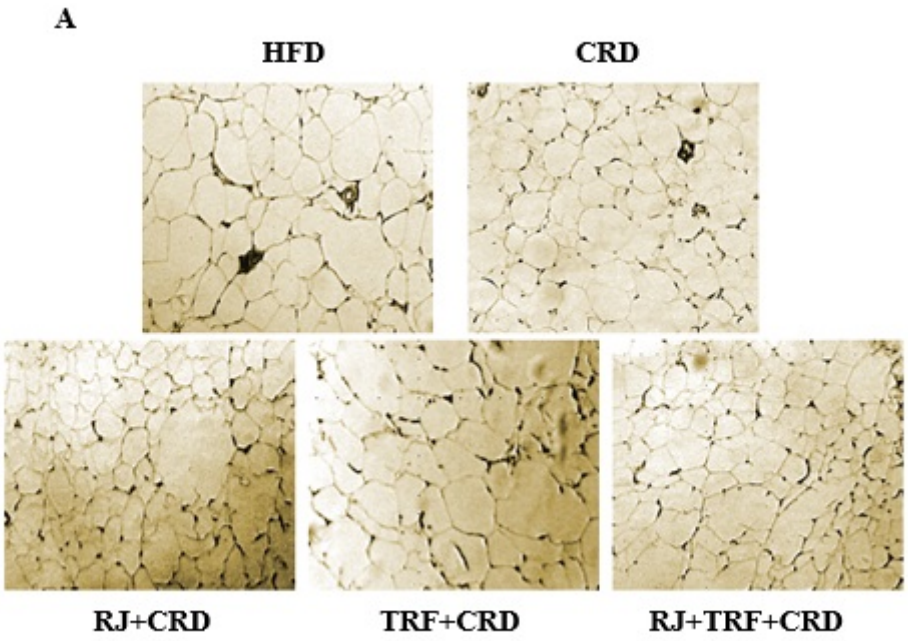

Figure 5

A) Representative images of H&E staining in sections of inguinal WAT of HFD, CRD, RJ, TRF and RJ+TRF rats;

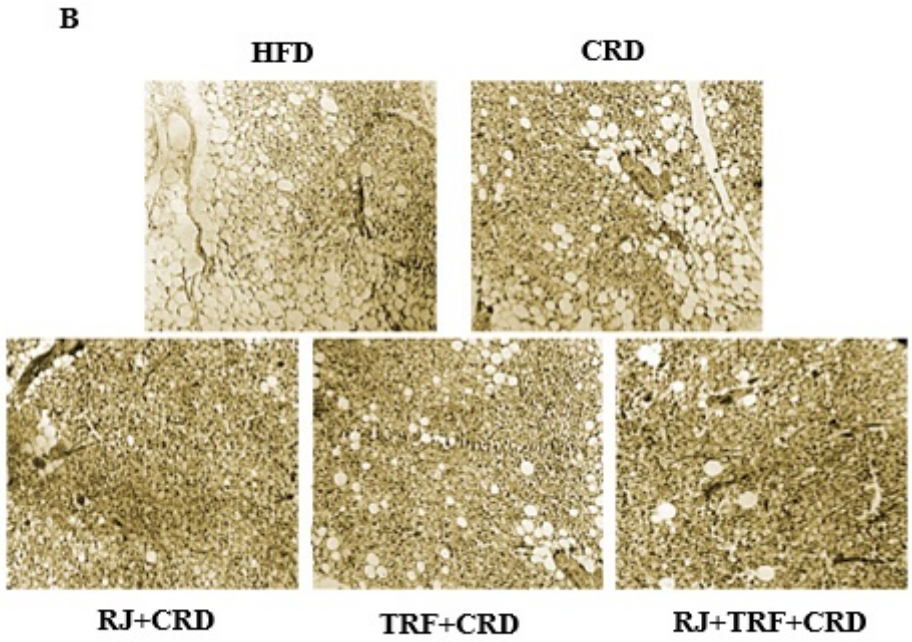

Figure 6

(B) Representative images of H&E staining in sections of interscapular BAT of HFD, CRD, RJ, TRF and RJ+TRF rats. All images were obtained at × 400 magnification

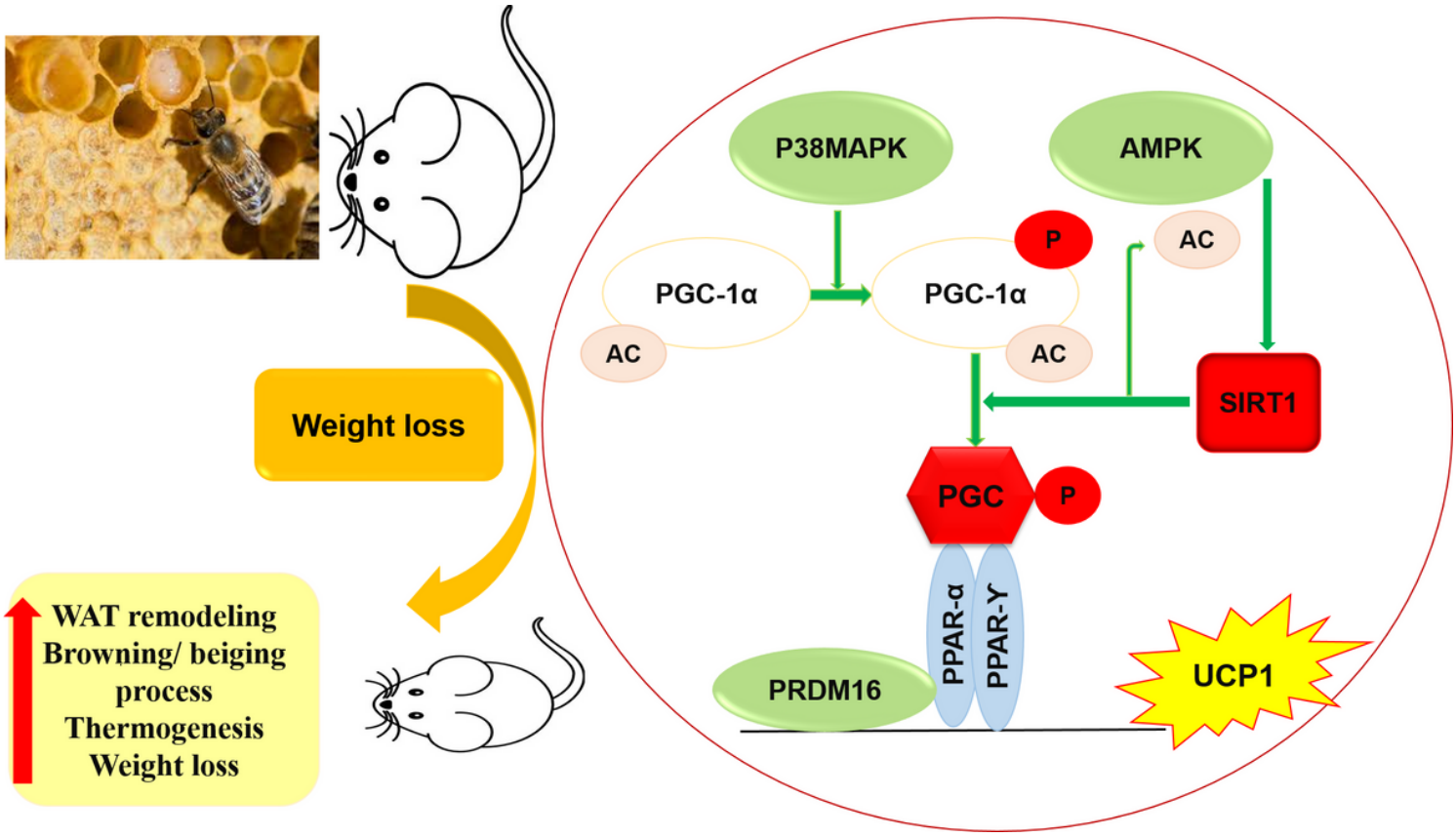

Figure 7

Suggested pathway contributing to adipose tissue remodelling and BAT activation and forming PGC1-α/PPAR/PRDM16 comlex following RJ consumption. P38 MAPK: P38 mitogen-activated protein kinase; AMPK: adenosine monophosphate-activated kinase; PGC1-α: peroxisome proliferator-activated receptor-γ coactivator 1α; SIRT1: sirtuin1; PPAR-α/γ: peroxisome proliferator-activated receptor-α/γ; PRDM16: PR domain containing16; UCP1: uncoupling protein 1; WAT: white adipose tissue