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Integrative Medicine Research

journal homepage: www.imr-journal.com

Original Article

The combination of exercise training and *Zataria multiflora* supplementation increase serum irisin levels in postmenopausal women

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ARTICLE INFO

Article history:
Received 9 October 2017
Received in revised form
4 December 2017
Accepted 22 January 2018
Available online 31 January 2018

Keywords:
Antioxidants
Circuit resistance training
Myokine
Postmenopausal women
Zataria multiflora

ABSTRACT

Background: We examined the effect of antioxidant supplementation and exercise on irisin within postmenopausal women.
Methods: Forty-eight participants (age: 55.7 ± 4.9 years; weight: 68.0 ± 6.3 kg; BMI 27.0 ± 2.7; mean ± SD) were randomized into four groups for the eight week intervention: control group (CG; $n = 12$), resistance training group (RTG; $n = 12$), supplementation with *Zataria multiflora* group (ZG; $n = 12$), or supplementation with *Z. multiflora* and resistance training group (ZRTG; $n = 12$). RTG and ZRTG performed circuit resistance training, and both ZG and ZRTG consumed 500 mg of *Z. multiflora* every day during the intervention. Blood samples were taken 48 hours before and after the intervention.
Results: There was a significant difference in irisin at post-training, with greater levels in ZRTG compared to CG. A significant increase was noted for irisin at post-training compared to pre-training for ZG, RTG, and ZRTG. Moreover, we identified a significant decrease in

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https://doi.org/10.1016/j.imr.2018.01.007
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malondialdehyde in the RTG and ZRTG groups and increase in glutathione in the ZG, RTG, and ZRTG groups when compared to CG.

Conclusion: These findings showed that exercise, *Z. multiflora* supplementation or their combination led to an increase in irisin.

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1. Introduction

In women, menopause-induced changes in hormones contribute to muscular atrophy, accelerated bone loss, and elevated oxidative stress, and consequently menopause may increase the physiological decline associated with aging and inactivity.[1–3]

Engagement in physical activity plays an important role in opposing physiological decline associated with aging and menopause.[4] A potential mechanism by which exercise may slow the effects of aging is by altering antioxidant status, an important component pathway in tissue damage and repair. Previously an acute exercise bout has been shown to increase oxygen consumption resulting in elevated generation of reactive oxygen species (ROS) in skeletal muscle.[5] Chronic exercise training on the other hand, may reduce inflammatory oxidative damage by increasing antioxidant capacity and reducing oxidative stress.[6]

In many cultures, certain plants are used as traditional medicines and ascribed anti-aging properties. Recently, there is a growing body of literature investigating the potential beneficial roles of many of these plants on physiological function including antioxidant capacity.[7] One such plant, which has important applications in traditional Iranian medicine, is *Zataria multiflora*, a plant belonging to the Lamiaceae family which contains various antioxidative components such as Thymol and Carvacrol.[7] Supplementation with *Zataria* extract has been shown to exhibit concentration-dependent radical-scavenging activity on 1,1-diphenyl-2-picryl-hydrazyl free radicals.[8]

Numerous exercise-induced myokines have been identified and recently, a newly discovered myokine, irisin has attracted significant attention.[9] Irisin is a peroxisome proliferator-activated receptor γ co-activator-1α (PGC-1α)-dependent and exercise-responsive myokine that stimulates browning of white adipocytes[10] and potentially has a role in antioxidant defense,[11–13] Boström et al. (2012) described expression of the irisin gene in skeletal muscle,[14] and aerobic exercise training has previously been reported to increase plasma irisin concentrations in post-menopausal women.[15]

To the best of our knowledge, the effects of plants with antioxidative properties such as *Z. multiflora* in combination with exercise training on the expression and activity of skeletal muscle myokines with roles in antioxidant status have not been investigated in postmenopausal women. Consequently, the current study examined the combined effect of *Z. multiflora* extract and circuit resistance exercise training (CRT) on the novel myokine irisin and antioxidant status (malondialdehyde, glutathione and total antioxidant capacity) in a group of post-menopausal women.

2. Methods

2.1. Study design

Following baseline testing, participants were matched based on weight, height, and body mass index (BMI), and then randomly divided into four equal groups by a person independent of the trial with group allocation provided in sequentially numbered opaque sealed envelopes. The four groups consisted of a control group (CG), who received eight weeks of usual care, a resistance training group (RTG), who received an eight week supervised circuit resistance training program, a *Z. multiflora* group (ZG) who received daily supplementation with a *Z. multiflora* supplement for eight weeks, and a *Z. multiflora* and resistance training group (ZRTG) who received both the eight-week circuit resistance training program and daily *Z. multiflora* supplementation. A number of studies have previously demonstrated resistance training to be a safe and effective exercise modality in post-menopausal women.[16–18] Ethics approval was obtained through the local Education Ethical Research Committee (Number: 4115), and the study was conducted in accordance with the Declaration of Helsinki.

2.2. Participants

Women were eligible for inclusion if they were at least six months' post-menopause (as confirmed by a gynecologist), had no addiction to drugs or alcohol, had no recent participation (last 6 months) in a planned exercise program, no history of renal, hepatic, cardiovascular disease, diabetes, and/or any physical injury or problem preventing participation in an exercise program. Postmenopausal status was confirmed by postmenopausal levels of serum estradiol (<120 pmol/L) and follicle-stimulating hormone (FSH > 30 IU/L).[19,20] Participants were advised that no new exercise should be commenced and not to use non-prescription medications and supplements during the trial. Before participating in the study, all procedures were explained to volunteers and after complete awareness of the study terms, and the completion of a medical questionnaire, written informed consent was obtained.

2.3. Zataria essential oil preparation and supplementation

Z. multiflora leaves were collected in March around the gardens of Eghlid, Shiraz. Then, they were dried in the shade for 10 days. Following drying in the shade the *Z. multiflora* leaves were dried in an oven for 48 hours at a temperature of 32°C and then powdered using a Chinese pounder. Fifty grams of the sample powder was extracted for 3 hours using water distillation with

a Clevenger apparatus at 100°C. The extract was filtered, then dried on anhydrous sodium sulfate, and finally transferred into a sealed glass container and stored at 4°C. The extract yield was calculated as dried oil volume divided by the initial dry powder weight multiplied by 100. The calculated yield was 3%.

2.4. *Zataria essential oil combination by gas chromatography–mass spectrometry (GC–MS)*

GC–MS was used to isolate and identify the components of *Z. multiflora* essential oil. GC–MS analysis was performed using Agilent 5975 mass spectrometer detector (MSD) coupled with gas chromatography model of Agilent USA GC 7890A MS 5975C. A column of welded silica HP-5 (5% phenyl, 1.95% polydimethyl siloxane) with profile of $30 \times 0.25\,mm^2$ and film thickness of 0.25 μm was used. Helium was used as the carrying gas and the flow rate of gradient was 1 mL/min. The thermal program used was as follows: first column temperature was set at 50°C for 5 minutes, then increased at 3°C/minute to 240°C, then increased at 5°C/minute to 300°C, and finally set for 3 minutes at this temperature. Essential oil samples were diluted by n-hexane at a ratio of 1:10 and 1 μL of the resultant solution was then injected into the gas chromatograph. The temperature at the injector and detector was fixed at 290°C. Compounds in *Z. multiflora* essential oil were identified using the fragmentation pattern in the database of wiley7n.land NIST08 and also using retention time in the chromatography column. For each combination, the ratio of the level below peak was determined to the total levels below peak of all compounds and the results are summarized in Table 1.

For supplementation, 500 mg of dry *Z. multiflora* leaves and powder were cast in capsules using a Chinese oven. Both ZG and ZRTG consumed 500 mg of *Z. multiflora* every day of the eight-week intervention. This consisted of one capsule (500 mg) with 100 mL of water after breakfast. RTG and CG consumed placebo capsules (500 mg wheat flour) with 100 mL of water after breakfast.

2.5. *Resistance training*

Following the familiarization week which involved training participants in correct lifting techniques, one repetition maximum (1RM) of the prescribed movements was determined using the Brzycki equation.[21] Training sessions were delivered using resistance circuit format with alternation between upper-body and lower-body movements as well as multi-joint movements. The exercises included: 1, squat; 2, chest press; 3, leg press; 4, standing military press; 5, knee extension; 6, seated cable rowing; 7, knee curl; 8, biceps curl, 9, standing calf raise; 10, triceps press; 11, back extension, and 12, abdominal crunch. The participants in both exercise training groups performed the movements at 55% of 1RM for eight weeks (3 sessions/week). Each exercise session included a 5 minutes warm-up and then followed with the 12 exercises. Each exercise was performed for a duration of 30 seconds, following which participants moved smoothly to the next exercise without a rest. The number of repetitions at each station was recorded for the participants. In each session, two sets of the 12 exercises were carried out with a 3 minutes active rest between each set.[22]

Table 1 – Compounds of *Zataria multiflora*

Compounds	Inhibition time (min)	Under peak level (%)
Thymol	35.90	26.8
Carvacrol	36.50	22.9
p-Cymene	21.73	7.7
γ-Terpinene	23.55	6.8
α-Pinene	16.53	3.2
β-Caryophyllene	41.36	3
Carvacrol methyl ether	32.86	2.4
α-Terpinene	21.12	2.2
Spathulenol	47.96	2
Linalool	25.59	1.8
β-Myrcene	19.60	1.5
Total	–	80.3

2.6. *Blood sampling*

Participants were requested to comply with the following conditions prior to each blood sample: 1) avoid use of non-prescription medications and supplements, 2) avoid any strenuous exercise other than the exercise prescribed as part of the study for at least 72 hours before the test, 3) to avoid coffee, dark tea, bananas, cereal and heavy or greasy foods at least 24 hours before the test, and 4) to match their diet in the 48 hours prior to each blood sample. Blood samples were taken following a 12-hour overnight fast a minimum of 48 hours before the first exercise session and 48 hours after the last exercise training session.

2.7. *Dietary analysis*

Participants were requested to complete a two-day food diary prior to both the baseline and endpoint blood sampling. Previously, a single-day of dietary analysis has been considered sufficient to assess compliance to dietary advice.[23] The amount of nutrients consumed was calculated using previously described methods.[23,24]

2.8. *Irisin measurement*

Human serum irisin levels were measured by sandwich ELISA as per manufacturer's instructions (CUSABIO, product number: CSB-EQ027943HU). The assay has high sensitivity (minimal detection dose less than 0.78 ng/mL) and specificity with no significant cross-reactivity between human irisin and analogs. Briefly, serum samples were left to coagulate at room temperature and centrifuged at $3000 \times g$ for 15 minutes. One hundred microliters of sample and standards were loaded per well and the optical density was determined at 450 nm, and corrected for optical plate imperfections at 540 nm.

2.9. *Antioxidant measurement*

Malondialdehyde (MDA) was measured using the thiobarbituric acid method as previously described[25] with spectrophotometer (Apel, Japan). Total glutathione (GSH) was measured

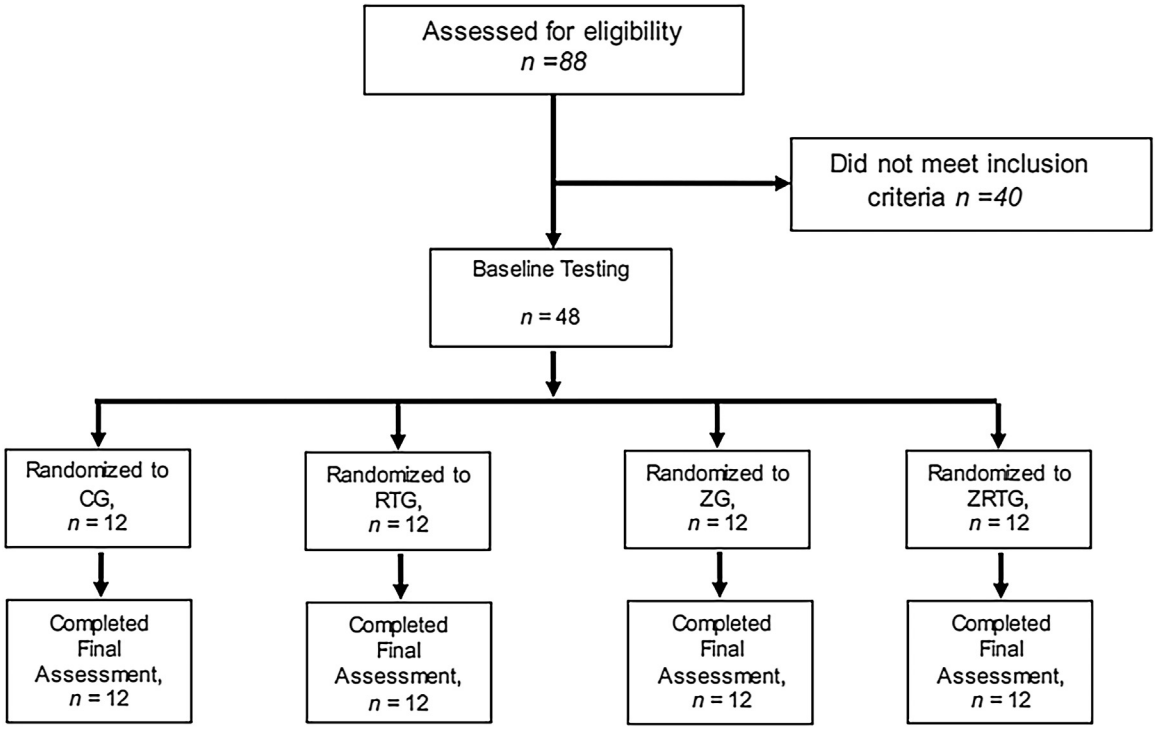

Fig. 1 – CONSORT flow chart.

utilizing an enzymatic recycling method as per manufacturer's instructions (Cayman Chemical Company, USA, Catalog number 703002). Briefly, serum samples were deproteinated, and GSH was measured by oxidation to produce 5-thio-2-nitrobenzoic acid (TNB) which was read colorimetrically at 405–414 nm on a plate reader (Biohit, Finland).

Total antioxidant capacity (TAC) was determined through ferric reducing/antioxidant power (FRAP) as previously described.[26] Briefly, antioxidants present in serum reduce the Fe^{3+}/tripyridyltriazine complex to blue colored ferrous form with an increase in absorbance at 593 nm. The change in absorbance is proportional to the combined FRAP value of the antioxidants in the sample.

2.10. *Statistical analysis*

All data were analyzed using software SPSS Version 20. The normality of data was approved by Kolmogorov–Smirnov test. Repeated measures analysis of variance (ANOVA) (4×2) was used to compare data of the four groups with *post hoc* analyses conducted using the Bonferroni method to locate means that were significantly different. Statistical significance was considered at the level of $p < 0.05$.

3. Results

Forty-eight participants (age: 55.7 ± 4.9 years; weight: 68.0 ± 6.3 kg; BMI 27.0 ± 2.7; mean $\pm$ SD) who met the inclusion criteria, consented to participate and underwent baseline testing (Fig. 1). Participants were randomly allocated to each of the four intervention groups: CG ($n = 12$), RTG ($n = 12$), ZG ($n = 12$), ZRTG ($n = 12$) and all participants completed the assigned intervention. Demographic characteristics of the participants at baseline and endpoint are provided in Table 2. No significant differences were noted in any demographic parameter between groups at baseline. No significant changes were noted for any demographic parameters in the CG. Fat mass significantly decreased at post-intervention in the ZG. Body weight, BMI, and fat mass significantly decreased at post-intervention while muscle mass increased in the RTG and ZRTG. Significant differences, however, were noted between groups at endpoint, with lower values for BMI in the ZG and RTG, and lower values for fat mass in the ZG, RTG, and ZRTG, when compared to the CG. Further, muscle mass was significantly higher in the ZRTG when compared to the ZG. Further, there were no differences within or between treatment groups in dietary intake in the two days prior to baseline or endpoint blood sampling (Table 3).

3.1. *Irisin measurement*

No significant differences in serum irisin levels were noted between the groups at baseline ($p = 0.12$). There was a significant difference in the response to the intervention between the groups ($p < 0.05$), with a significant increase in ZRTG when compared to the CG ($p < 0.05$). However, no significant differences were measured in the change between the other groups (ZG and RTG) ($p > 0.05$). In addition, a significant increase was noted from pre- to post-supplementation for ZG ($p < 0.01$, 3.09%), RTG ($p < 0.001$, 4.14%), and ZRTG ($p < 0.001$, 8.07%) (Fig. 2).

Table 2 – Demographic Characteristics of Participants at Baseline and Endpoint (Mean ± SD).

Groups	CG		ZG		RTG		ZRTG	
	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint	Baseline	Endpoint
Age (years)	56.5 ± 4.2	–	54.4 ± 3.9	–	58.03 ± 4.7	–	53.8 ± 6.0	–
Height (cm)	156.7 ± 3.0	–	160.9 ± 4.0	–	158.7 ± 3.8	–	159.2 ± 4.6	–
Weight (kg)	68.7 ± 13.3	68.7 ± 4.7	66.4 ± 10.9	65.9 ± 5.1	67.1 ± 7.2	64.3 ± 5.6*	69.9 ± 5.7	67 ± 4*
BMI (kg/m^2)	27.9 ± 2.2	27.9 ± 1.8	25.6 ± 2.2	25.4 ± 1.6#	26.6 ± 3.1	25.5 ± 2.3*#	27.6 ± 2.7	26.4 ± 2.1*
Fat mass (%)	20.6 ± 1.7	20.5 ± 1.6	19.9 ± 1.9	18.6 ± 1.4*#†	20.1 ± 2.1	17.1 ± 1.9*#	21 ± 1.4	18 ± 1.1*#
Muscle mass (kg)	27.6 ± 2.5	27.6 ± 2.3	26.5 ± 2.5	26.8 ± 2.1	26.8 ± 2.8	28.4 ± 2.2*	28 ± 1.9	29.1.9*

Data are expressed as mean ± SD. BMI – body mass index, CG – control group, ZG – *Zataria* group, RTG – resistance training group, ZRTG – *Zataria* and resistance training group; *$p < 0.05$ vs. baseline in same group; #$p < 0.05$ vs. CG at endpoint, †$p < 0.05$ vs. ZRTG at endpoint.

Table 3 – Food Intakes (Mean ± SD) of Study Groups for 2 Days Before the Pretest and Posttest Blood Sampling.

Groups	CG		ZG		RTG		ZRTG	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Total calorie (kcal/d)	2225 ± 190	2396 ± 103	2203 ± 157	2317 ± 98	2142 ± 125	2276 ± 180	2096 ± 113	2162 ± 125
Total protein (g/d)	111 ± 16	112 ± 17	112 ± 14	110 ± 18	110.5 ± 17	108 ± 19	104 ± 16	102 ± 19
Protein (g/kgBW/d)	1.05 ± 0.45	1.2 ± 0.4	1.1 ± 0.4	1.0 ± 0.3	1.0 ± 0.3	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.2
Total protein (% energy)	3.18 ± 4.1	19.6 ± 3.5	18.4 ± 4.2	19.7 ± 4.9	19.5 ± 4.6	19.0 ± 4.2	20.9 ± 4.8	19.3 ± 3.8
Total carbohydrate (g/d)	295 ± 19	304 ± 23	285 ± 20	289 ± 25	248 ± 18	250 ± 24	252 ± 17	245 ± 22
Total carbohydrate (% energy)	48.5 ± 6.9	50.8 ± 7.1	47.4 ± 7.2	50.5 ± 7.0	45.5 ± 9.8	49.0 ± 7.2	45.7 ± 10.1	49.3 ± 7.3
Total fat (g/d)	81.5 ± 21	77.3 ± 27	83.5 ± 23	78.0 ± 26	77.0 ± 22	71.0 ± 25	70.0 ± 17	66.0 ± 19
Total fat (% energy)	31.9 ± 7.6	28.8 ± 6.9	32.5 ± 6.9	29.0 ± 6.4	32.6 ± 7.0	31.4 ± 6.5	31.8 ± 9.2	30.1 ± 6.3

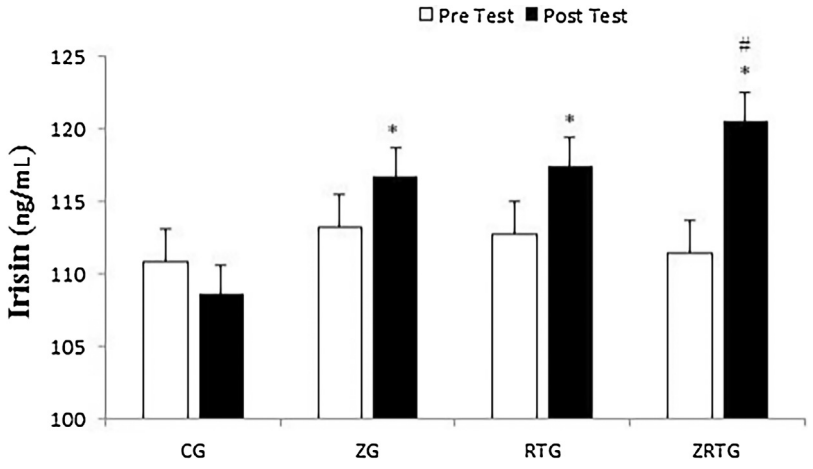

Fig. 2 – Serum irisin in pre- and post-test. Data are expressed as means ± SD. *$p < 0.05$ versus pretest in same group. #$p < 0.05$ versus CG.

3.2. *Antioxidant status*

No significant differences in MDA, GSH or TAC were measured at baseline between the CG, ZG, RTG or ZRTG (Table 4). However, a significant decrease in MDA levels were observed in the RTG and ZRTG groups ($p < 0.05$ and $p < 0.001$, respectively). Similarly, a significant increase in GSH was measured for the ZG ($p = 0.001$), RTG ($p < 0.01$) and ZRTG ($p < 0.001$). There was no significant difference in TAC between groups (Table 4).

4. Discussion

Our major findings were that irisin levels significantly increased following eight weeks of combined circuit resistance training and supplementation with *Z. multiflora* compared to the CG in apparently healthy post-menopausal women. Further findings were that resistance training, supplementation with *Z. multiflora* and the combination of resistance training and supplementation resulted in significant increases in irisin levels within the groups. MDA levels were significantly reduced in the RTG and ZRTG when compared to the CG; however MDA was not affected by *Z. multiflora* alone. GSH levels were significantly increased in the ZG, RTG, and ZRTG when compared to the CG. TAC did not change during baseline or the intervention.

It has been reported that an acute bout of exercise increases oxidants and decreases antioxidants pushing the balance toward oxidative stress.[27] In contrast chronic resistance exercise training has been reported to provide protective effects

Table 4 – Antioxidant Results (Mean ± SD) of Study Groups at Baseline and Endpoint

Variable	Group	Baseline	Endpoint	MD	95%CI	Sig (*p*)	MD	95%CI	Sig (*p*)	MD	95%CI	Sig (*p*)
MDA	CG	1.62 ± 0.28	1.54 ± 0.17									
(nmol/mL)	ZG	1.65 ± 0.45	1.16 ± 0.21	−0.18	−0.40 to 0.05	0.203						
	RTG	1.56 ± 0.44	1.08 ± 0.19	−0.26	−0.49 to −0.04	0.013	−0.08	−0.31 to 0.14	1			
	ZRTG	1.54 ± 0.22	0.87 ± 0.14	−0.38	−0.60 to −0.15	0.000	−0.20	−0.42 to 0.02	0.110	−0.12	−0.34 to 0.11	1
GSH	CG	237 ± 23	230 ± 24									
(mg/mL)	ZG	246 ± 12	267 ± 19	23.06	7.32 to 38.79	0.001						
	RTG	231 ± 22	279 ± 19	21.85	6.11 to 37.59	0.002	−1.21	−16.9 to 14.5	1			
	ZRTG	241 ± 23	282 ± 17	28.49	12.75 to 44.22	0.000	5.43	−10.3 to 21.2	1	6.64	−9.10 to 22.37	1
TAC	CG	520 ± 101	493 ± 77									
(mmol/L)	ZG	510 ± 79	562 ± 84	29.9	−36.2 to 95.9	1						
	RTG	506 ± 110	566 ± 90	30.1	−36.0 to 96.1	1	0.2	−65.8 to 66.2	1			
	ZRTG	500 ± 71	627 ± 52	57.0	−9.0 to 123.0	0.133	27.1	−38.9 to 93.2	1	26.9	−39.1 to 93.0	1

Data presented as mean ± SD. MDA denotes malondialdehyde, GSH denotes glutathione, TAC denotes total antioxidant capacity.

against oxidative stress[28] while eight weeks of combined endurance and resistance training decreased oxidative stress and increased enzymatic and non-enzymatic antioxidant capacity in untrained males.[29] In this context, we measured significant changes in the antioxidants GSH and MDA (but not TAC) in response to exercise and *Z. multiflora* supplementation. GSH levels increased in the ZG, RTG and ZRTG when compared to the CG, while MDA levels decreased in the RTG and ZRTG, but not in the ZG alone.

GSH plays an important role in countering the oxidative-stress induced lipid peroxidation, which occurs during intense exercise.[30] The increase in GSH in the RTG suggests an adaptation of the tissue-antioxidant defense system in response to exercise in these participants.[31] An increase in GSH by *Z. multiflora* in the ZG alone suggests that this plant potentially has a protective effect against oxidative stress. This study corroborates with similar research where *Z. multiflora* showed strong biological antioxidant activity, and nitric oxide and malondialdehyde scavenging properties in chemical analysis.[32] Moreover, recent studies in male rats evaluated the protective effects of *Z. multiflora* on cisplatin-induced hepatotoxicity and hepatic oxidative damage [33]. Rats given a methanolic extract from *Z. multiflora* orally for seven days had increased levels of GSH that reduced cisplatin-induced oxidative stress. Similarly, studies in adult mice assessing the effect of *Z. multiflora* on hyperglycemia and blood glucose levels, lipid profile, and oxidative stress status reported significant decreases in glucose, total cholesterol, and MDA that were concomitant with an increase in GSH levels.[34]

We observed a significant reduction in the levels of MDA in the RTG and ZRTG, but not in the ZG alone. MDA is a common marker of lipid peroxidation and oxidative stress and levels of MDA are reported to increase following acute intense exercise.[35] These data suggest that chronic exercise may have a greater advantage in protection against the effects of oxidative stress. These findings corroborate with Çakir-Atabek et al. (2010) where chronic resistance exercise training in men resulted in significantly reduced MDA levels, increased GSH levels, and therefore suggests a protective mechanism against oxidative stress.[28] It is unclear why ZG alone did not reduce the levels of MDA, but it is interesting that the mean decrease in MDA in ZG was approximately 75% of that observed in the RTG group, and that MDA in the ZRTG group decreased by 25% more than RTG alone. It is possible therefore, that the lack of significance in the ZG group and in ZRTG compared to RTG was due to a smaller relative effect of *Z. multiflora* on antioxidant status than exercise, and that these differences would have become statistically significant with a larger sample population. This needs further investigation in future studies.

Our analysis of TAC found no significant changes between any of the groups; however, a trend was observed where TAC increased in the ZG, RTG and ZRTG, which may reflect enhanced antioxidant defenses in response to exercise-induced oxidative stress. This finding is in accordance with previous studies that showed increased plasma TAC levels across a range of exercise modalities.[36–38]

A potential mechanism by which these protective benefits may occur are through increased production and release of muscle-derived secretory factors or myokines. Myokines facilitate tissue-specific crosstalk between skeletal muscle and other tissues such as liver and adipose tissue.[39] Myokines possess important anti-inflammatory and metabolic properties and play a crucial role in muscle adaptation to exercise.[40] Previously irisin was defined as a myokine that is responsive to exercise,[41,42] negatively correlated with malondialdehyde,[13] and improves endothelial dysfunction via decreasing oxidative stress in type 2 diabetes.[43]

Nevertheless, several studies have failed to show increases in irisin levels in plasma after chronic training,[44,45] and reduced plasma levels of irisin after intermittent sprint running has also been demonstrated.[44] Norheim et al. (2014) reported that circulating irisin was reduced in response to 12 weeks of training, but was increased after acute exercise in men.[41] Conversely, others reported that acute strength training for up to 30 minutes did not increase serum levels of irisin.[45] Interestingly, the only study to our knowledge to previously examine the effect of exercise on irisin levels in post-menopausal women found similar results to the current study following an eight-week aerobic exercise intervention.[15] The reasons for these contradictory results are not known but may relate to differences in the cohort studied.

Irisin levels significantly increased following *Z. multiflora* supplementation. Previously *Zataria* has been shown to exhibit radical-scavenging activity and protection of human lymphocytes from the genetic damage and side-effects

induced by exposure to radiation.[8] Therefore, we believe the increases in irisin levels following eight weeks of supplementation with *Z. multiflora* are due in part to positive effects of *Z. multiflora* on antioxidative enzyme activities, resulting in reduced oxidative stress, mainly through promoting enzymatic and non-enzymatic antioxidant capacity. These data support previous work indicating a relationship between irisin and antioxidant capacity.[11,13] Whether irisin is a vital molecule involved in up-regulation of antioxidant capacity or the increase is secondary to improved antioxidant capacity, is an important issue, which should be considered in future research. Similarly, it is not clear how *Z. multiflora* increased irisin levels, and no reports to our knowledge exist on the mechanism(s) by which this might occur. However, as previously noted, irisin can be activated by PGC-1α[10], and this transcriptional coactivator can activate peroxisome proliferating activator receptor gamma (PPARγ).[46] Previously, research demonstrated that *Z. multiflora* had a direct insulin-like effect, increased adiponectin, and activated PPARγ protein expression which was concomitant with improved anti-hyperglycemic effects and improved insulin sensitivity in insulin-resistant rats.[47] Accordingly, it is an intriguing notion that perhaps *Z. multiflora* could act through the PGC-1α/PPARγ transcriptional network to increase irisin levels. However, this is yet to be tested.

Another novel finding of the current study was that serum levels of irisin significantly increased after eight weeks of combined circuit resistance training and supplementation with *Z. multiflora*. However, increases in irisin levels in the combined treatment were not significantly greater than either the exercise or the herbal treatment alone, indicating no additive benefits in post-menopausal women. Our findings are supported by those of a previous study[48] who failed to find any positive additive effect of combined antioxidant supplementation and exercise training on cognitive function.[48] There are, however, some contradictory findings regarding positive additive effects of combined antioxidant supplementation and exercise training on various body organs and/or systems. In animals, an additive effect of combined antioxidant supplementation (i.e., vitamin E and Omega-3 fatty acids) and exercise training (i.e., swimming) has previously been observed to increase antioxidant activity in brain tissue[49] and improve synaptic plasticity[49,50] which is inconsistent with findings of the current study. As mentioned earlier, both exercise training (chronic) and *Z. multiflora* have been reported to promote antioxidant capacity. Therefore, the combination of antioxidant supplementation and exercise training is expected to boost antioxidant capacity and potentially could lead to a greater improvement than either intervention separately. The lack of an additive effect on irisin levels in the current study may be due in part to reaching a maximum ceiling of antioxidant capacity during each intervention. Further studies are needed to confirm these findings.

It is important to note the limitations of the study. Although compounds from *Z. multiflora* have been used in several studies, we do not have the information to determine exactly which compounds (or combination thereof) are causing the observed effects. Similarly, while we measured irisin in serum, it is not clear whether the irisin we measured was produced and secreted from skeletal muscle. It is tempting to speculate that the irisin measured in the current study is muscle-specific based on studies by Boström et al. (2012) that showed exercise induced the expression of PGC-1α that subsequently increased irisin expression and secretion from skeletal muscle cells.[14]

Another potential limitation is that related factors to myokines, such as insulin, brain-derived neurotrophic factor, and Interleukin-6 were not assessed in this study. Additionally, while ELISA is widely used to detect and quantify biological molecules, it cannot be ruled out that non-specific cross-reactivity with other proteins might occur. The specificity information provided with the ELISA kit (CUSABIO) indicated high and excellent sensitivity for detection of human irisin and has been reported previously.[51–54] Ideally, it would be good practice to confirm non-specific cross-reactivity in these studies by immunoblotting for the expected molecular weight of irisin and/or the use of recombinant irisin and size comparison. Unfortunately, there was insufficient sample available to perform these additional studies in this case and consequently the potential for cross-reactivity must be acknowledged as a potential confounder.

The present study provided some evidence of positive effects of circuit resistance training as well as *Z. multiflora* supplementation on the expression of the novel myokine, irisin, within post-menopausal women presumably through antioxidant pathways. The interaction between the expression of myokines and antioxidant pathways need to be the subject of more detailed studies.

Conflict of interest

The authors declare no conflict of interest.

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