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Impact of saffron (Crocus Sativus Linn) supplementation and resistance training on markers implicated in depression and happiness levels in untrained young males

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ABSTRACT

Background: We aimed to assess the effects of six weeks of resistance training (RT) combined with saffron supplementation on markers implicated in depression as well as happiness levels in untrained young males.
Materials and methods: Untrained young male participants were randomly assigned to one of two groups: RT + saffron supplement (RS; $n = 14$) or RT + placebo (RP; $n = 14$). For 6 weeks, participants in the RS group took one 150 mg pill of pure saffron immediately after each RT session and at the same time on non-training days. Those assigned to the RP group took a dextrose pill. Concentrations of Anandamide (AEA), 2-Arachidonoylglycerol (2-AG), serotonin, dopamine, β-endorphin (beta-endorphin), tryptophan, happiness levels (via questionnaire), and body composition were assessed before and after the 6 weeks of whole-body supervised RT (4x/week, 3 sets using 60–70% of 1-repetition maximum [1-RM]).
Results: AEA (0.5 ng/ml), 2-AG (0.04 ng/ml), dopamine (0.7 ng/ml), and β-endorphin (9.4 pg/ml) concentrations significantly increased in the RS group ($P<0.05$) while no changes were detected in the RP group. Serotonin (RS = 1.7 ng/mL and RP = 1 ng/mL) concentrations and happiness levels significantly increased in both groups with greater changes in RS group while tryptophan concentrations remained unchanged ($P> 0.05$). In addition, both groups significantly increased muscular endurance with greater changes in RS group ($P<0.05$).
Conclusion: Six weeks of RT combined with saffron supplementation improved AEA, 2-AG, dopamine, β-endorphin, and serotonin concentrations. Moreover, the addition of saffron supplement to chronic RT results in greater improvements in happiness levels than RT alone.

1. Introduction

It is well known that resistance training (RT) is the most prominent training intervention to generate muscle gain (e.g. hypertrophy, strength, endurance, etc.) in various populations [1-3]. Other than muscle gain, RT has also been shown to improve mental well-being through reductions in depressive moods, tension, and anxiety [4-6], as well as increasing levels of happiness [7]. This was underlined in a recent meta-analysis of 33 randomized controlled trials (RCTs) featuring RT which reported significant reductions in depressive symptoms and improvements in the levels of happiness [8]. In addition to RT, some herbs, such as saffron (Crocus Sativus Linn), have received considerable attention due to their antidepressant properties which enhance mental well-being [9]. More precisely, saffron supplementation may inhibit the reuptake of serotonin in synapses, thus increasing brain exposure and thereby improving its positive effects [10]. Indeed, a meta-analysis

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confirmed saffron's beneficial effects on depression in adults with major depressive disorders [11].

Although the anti-depressive effects of both saffron supplementation and RT are well known, the effect of the combination of these two interventions on markers implicated in depression and levels of happiness is less studied in humans. The addition of a nutritional strategy to RT may be essential to positively affect mental well-being and potentially create an additive effect not achieved by each intervention alone. To the best of our knowledge, there is no reliable evidence for the effects of RT combined with saffron supplementation on markers implicated in depression and happiness levels. Therefore, we conducted this study to estimate the effects of 6 weeks of RT combined with saffron supplementation (150 mg/d of pure saffron) on Anandamide (AEA), 2-Arachidonoylglycerol (2-AG), serotonin, dopamine, β-endorphin (beta-endorphin), and tryptophan concentrations, as well as happiness levels (via questionnaire), in untrained young males. Previous studies have evaluated a dose of 500 mg/d for two weeks [12] or 300 mg/d for 10 days [13]. As we aimed to evaluate a longer-term study compared to those previously performed, a pharmacologist prescribed a reduced dosage (150 mg/d) to prevent any potential side effects. Finally, we hypothesized that 6 weeks of RT combined with saffron supplementation would improve the stated markers' concentrations as well as happiness levels in untrained young males.

2. Materials and methods

2.1. Participants

Thirty untrained young males (age = 24 ± 2 years; stature = 176.4 cm ± 3.5 cm) were recruited via telegram channels and word of mouth to participate in this study. None of the participants regularly exercised (<1 hour/week) and had no prior RT experience. The inclusion criteria for the study were not taking dietary supplements, medications, consuming alcohol, or smoking during the year prior to enrollment in the study, being healthy (no known medical issues such as diseases, diabetes, sleep disorders, or other risk factors), and not having an allergy/sensitivity to saffron. Failure to meet any of these criteria excluded the potential participants from enrolling. Exclusion after the initiation of the study occurred if participants were unwilling to undertake the nutritional or RT protocol, participated in exercise other than the prescribed RT program throughout the length of the investigation, consumed any dietary supplements, and missed more than one RT session or post-RT saffron supplementation during the study period. All these criteria were evaluated by a physician using the Physical Activity Readiness-Questionnaire (PAR-Q) and the medical health/history questionnaire. Participants gave written informed consent prior to initiating the study. All experimentation was carried out in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the Iranian Institute of Physical Education and Sports Sciences and was retrospectively registered at the Iranian Registry of Clinical Trials (IRCT2017082534144N2).

2.2. Study design

A randomized, double-blind, and parallel design was used in the current study. Participants were familiarized with all testing and experimental procedures prior to baseline measurements. This consisted of 3 RT familiarization sessions with instruction on the proper lifting technique and acquaintance with all exercises and equipment. Participants were randomly assigned to either a RT+ saffron supplementation (RS; $n = 14$) or a RT+ placebo (RP; $n = 14$) group. The allocation was stratified by body mass index [(BMI) (<25.0 kg/m^2 or ≥25.0 kg/m^2)] and the sequence was randomized by a computer. An independent coordinator (not otherwise involved in data collection, data analysis, authorship, or other aspects of the study) assisted with group assignment duties. Both the participants and research team members were blinded to the treatment allocation until the database was unlocked and data analysis was completed. Measurements were taken at two-time points, baseline and after 6 weeks, at the same time of day (±1 hr) in the morning following an overnight fast and abstinence from caffeinated drinks and alcohol and approximately 48 hr after the last training session. Participants were instructed not to alter their regular lifestyle habits during the study and submit 3-day food records (at baseline and 6 weeks) to minimize dietary and physical activity variabilities. The schematic diagram of the study design is presented in Fig. 1.

2.3. Anthropometry and body composition

Upon arriving at the laboratory, participants were asked to void completely within 30 min of the test. Body mass (BM) was measured with a digital scale (SECA, Germany) to the nearest 0.1 kg. The participant's stature was measured with a stadiometer (SECA, Germany) to the nearest 0.1 cm. Body mass index (BMI), body fat percentage (BFP), and lean mass were evaluated by a multi-frequency bioelectrical impedance device (OLYMPIA 3,3 JAWON, South Korea) as previously described [14]. The test-retest reliability of the bioelectrical impedance method is high ($R = 0.95$ to 0.99) [15].

2.4. Blood sampling and laboratory analysis

Fasting blood samples (10 mL) were collected from the antecubital vein using standard procedures. Following completion of blood sampling, samples were centrifuged at 3000 rpm for 10 min, and serum was stored at −80 °C for future analysis of AEA (My BioSource, sensitivity: 0.1 ng/ml), 2-AG (My BioSource, sensitivity: 0.2 ng/ml), serotonin (My BioSource, sensitivity: 0.1 ng/ml), dopamine (CUSABIO, sensitivity: 0.2 ng/ml), β-endorphin (CUSABIO, sensitivity:15.6 ng/ml), and tryptophan (My BioSource, sensitivity:1 ng/ml) concentrations. The intra- and inter-assay Coefficient of Variation (CV) for AEA and serotonin were less than 10% and 10% respectively, less than 10% for both the intra and inter 2-AG assays, 8% and 10% for dopamine and β-endorphin respectively, and less than 9% and 10% for tryptophan.

2.5. Happiness assessment

The question ("In general, do you feel happy?") was used to measure the levels of happiness. The participants scored from zero to 10 using an analogous visual scale. The reliability and validity of this method have been previously described [16].

2.6. Muscular strength and endurance testing

Muscular strength testing was performed 24 h after the body composition, blood sampling, and happiness assessments with similar pre-participation requirements. Participants executed the 1-RM tests for all exercises used during RT in order to establish a training load. Participants were required to execute one set of 8–10 repetitions of light intensity as a warm-up for each exercise. After 3 minutes of rest, the load was increased until they were incapable of lifting the added weight. Complete range of motion, proper technique, and appropriate rhythm (2 s concentric and 2 s eccentric) were required for successful trials. All 1-RM values were determined within five attempts with rests of 3–5 min between each trial and 10 min between different exercises [17]. After 48 h, muscular endurance was assessed through a standard push-up test as previously described [18].

2.7. Resistance training

The RT program consisted of 24 RT sessions (6 weeks, 4x/week). The exercises included leg presses, leg curls, bench presses, lat pulldowns, biceps curls, and triceps pushdowns that were performed on variable resistance machines (Hoist, USA). Three sets with an intensity of 60% to

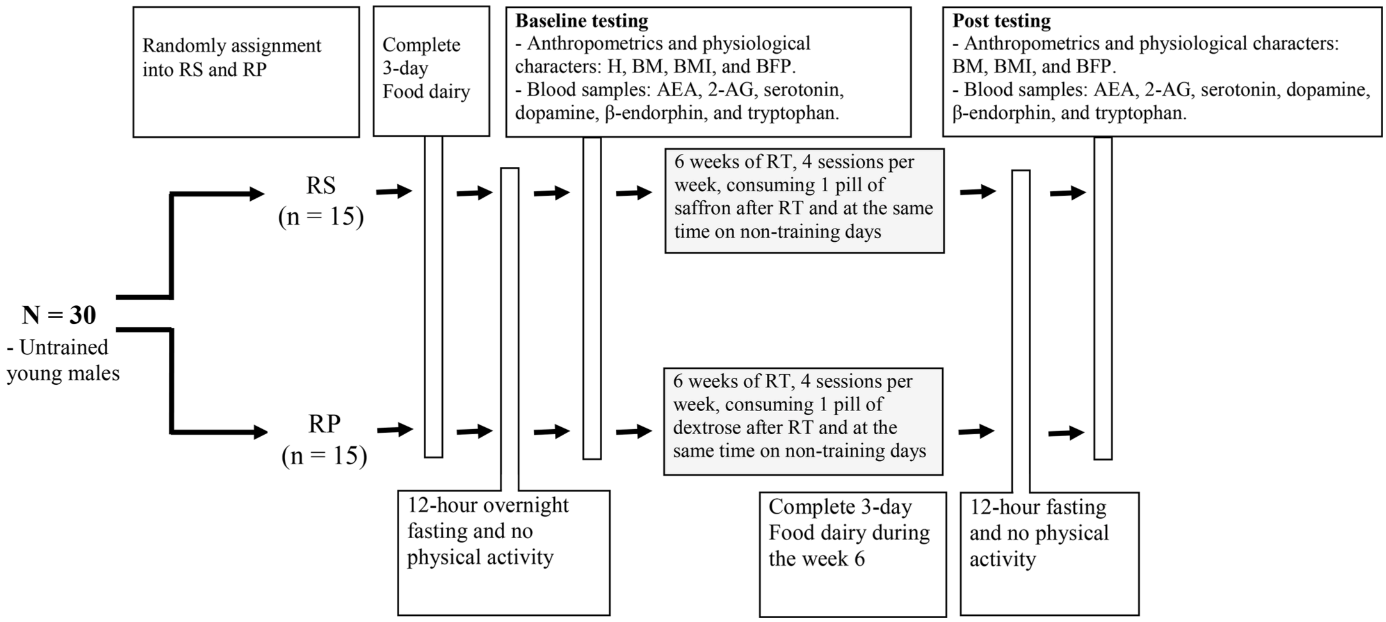

Fig. 1. The schematic diagram of the study design.

70% of 1RM were performed for each exercise. There were rest periods of 40–60 s between sets and 2 min between each exercise. In order to follow the overload principle and ensure gradual progress, 1RM testing was also performed after the 2nd and 4th week to update the exercise loads. At the beginning of each RT session, participants warmed up their bodies (both generally and with specific warm-ups) for 10 min. If a participant missed an RT session for any reason, a make-up session was held at the earliest opportunity (within a week). All RT sessions were conducted between 4 p.m. and 6 p.m. under the supervision of RT specialists. The periodized protocols were adapted from previous studies [1,3] and followed recommendations by the National Strength and Conditioning Association [19].

2.8. Saffron supplementation

Participants in the RS group received one 150 mg pill of pure saffron immediately after each RT session and at the same time on non-training days for 6 weeks. Likewise, the RP group consumed one pill of dextrose at the same time point to match the number of pills consumed by the RS group. Saffron tablets (pure head powder of natural saffron compressed and without any additives) were processed under the Hazard Analysis Critical Control Point (HACCP) health and food safety system and made with license number 10,133/20 from the Ministry of Health of Iran and were purchased from Tarvand (Iran). Briefly, after drying saffron leaves for 2 weeks, they were powdered. The isolation of the volatile compounds was performed using an ultrasound water bath sonorex at the fixed frequency of 35 kHz. After sonication for two times for 15 min, the organic extract was collected and concentrated by a gentle flow of nitrogen and a minor quantity of anhydrous magnesium sulfate was added. The metabolic content of this product per gram of saffron is about 19.7 mg of crocin and 0.25 mg of safranal. Placebo and saffron tablets were identical in appearance, taste, and smell. The selected dose and time of ingestion were based on prior research that revealed improved levels of markers implicated in depression after chronic saffron consumption in various populations [9]. It should be noted that researchers monitored supplement consumption. Following the RT sessions, the pill was given to the participants by a researcher who witnessed its consumption. On non-training days, the supplementation was monitored by phone call, WhatsApp, or Telegram software. It should be noted that all researchers and participants were blinded to the saffron supplementation.

2.9. Nutrient intake and dietary analysis

We monitored the dietary intake of participants in the hours either side (before and after) of the RT sessions since nutritional timing (during and around an exercise) plays a critical role in training adaptation [20]. Participants ate a banana (providing ~0.30–0.35 g of carbohydrate/kilogram of BM) as a pre-exercise snack approximately one hour before the RT sessions. Dinner was consumed ~1.5–2 h after each RT session and was standardized to contain 1.7 g/kg of BM of carbohydrate, 0.3 g/kg of BM of protein, and 0.4 g/kg of BM of fat [21], in accordance with recommendations for macronutrient distribution (55–65% of total calories from carbohydrates, ˂35% of total calories from fats and 10–15% of total calories from protein) as well as protein timing (~0.25 g to 0.3 g of protein/kg of BM within 2 h after RT) given by the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine [22]. In addition to this standardized pre-RT nutrient intake, participants were instructed not to alter their dietary habits during the study. Participants were asked to submit 3-day food records (2 weekdays and 1 day of the weekend) at baseline and near the completion of the assigned intervention. Each food item was individually entered into the Diet Analysis Plus program (version 10, Cengage, Boston, MA, USA) and the total energy consumption and the amount of energy derived from proteins, fats, and carbohydrates were assessed [21].

2.10. Statistical analysis

The estimation of an appropriate sample size was conducted using the G*Power analysis software. Our rationale for sample size was based on a previous study that observed significant changes in β-endorphin concentrations following RT in young males [7]. The analysis revealed that a sample size of at least 24 participants (n = 12 per group) was needed to provide power (1-β) of 0.8 (α = 0.05). The normality of data was confirmed using the Shapiro-Wilk test. Possible group differences at baseline were examined using a one-way analysis of variance (ANOVA). A repeated measures general linear model (2 × 2) was used to assess the effect of intra-subject factors (baseline vs. 6 weeks), inter-subject factors (i.e. training protocol: group), and the interaction effects (time × group). This was followed by the appropriate Bonferroni post hoc test when significant treatment and treatment-by-time interactions were revealed. Statistical significance was set at P <0.05. All statistical procedures were performed using SPSS (version 24.0, IBM; Chicago, IL) and all the figures were prepared in Graphpad Prism (version 8.0.2).

3. Results

Of the 36 male volunteers identified, six did not meet our initial inclusion criteria. Consequently, 30 participants were randomized into either the RS or the RP group. One participant was excluded from each group due to unwillingness to carry out our RT protocol. Data are therefore presented for the 28 participants that finished the study (n = 14 per group). Fig. 2 shows the flow diagram of the participants' progress through the study. Participant compliance in both groups (RS and RP) was approximately 94%. Compliance with both saffron and placebo supplements was also ~94%. There were no significant differences over time or between groups in the mean daily energy intake or the amount of protein, carbohydrates, and fats consumed per day (Table 1).

3.1. Markers implicated in depression

There were no significant between-group differences at baseline. A significant group x time interaction effect was observed for serotonin [(P = 0.021), (F = 6)] concentrations. In addition, a significant time effect was noted for AEA [(P = 0.006), (F = 9)], 2-AG [(P = 0.002), (F =

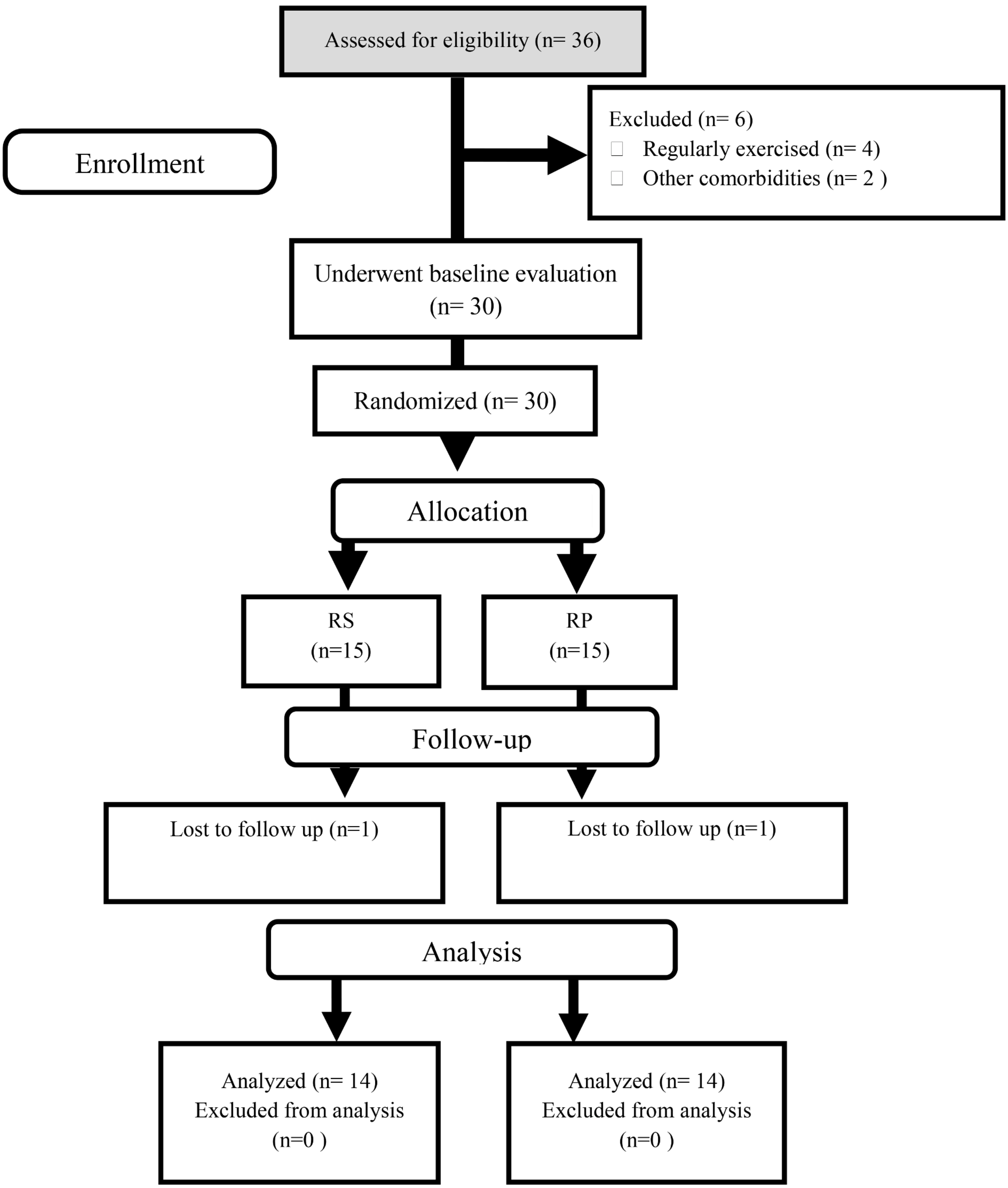

Fig. 2. Flow diagram of the participants' progress.

Table 1
Energy and macronutrients (mean ± SD).

Variables	Group	Pre-training	Post-training	P
Energy (kcal/day)	RS	1837.1 ± 60.6	1811 ± 50.9	0.085
	RP	1864.5 ± 84.6	1871.4 ± 76	
Protein (g/day)	RS	80.6 ± 4.4	82.7 ± 5.2	0.822
	RP	81.2 ± 4.6	83 ± 7.1	
Fat (g/day)	RS	56 ± 3.5	53.6 ± 3.1	0.087
	RP	59.1 ± 8.2	57.4 ± 6.3	
Carbohydrate (g/day)	RS	252.6 ± 8.1	251.2 ± 8	0.826
	RP	251.8 ± 6.5	253.2 ± 6.6	

Abbreviations: RS, Resistance Training + Saffron Supplementation; RP, Resistance Training + Placebo; kcal/day, kilocalorie/day; g/day; gram/day.

12.1)], β-endorphin [($P<0.001$), ($F = 22$)], tryptophan [($P = 0.042$), ($F = 4.5$)] and dopamine [($P<0.001$), ($F = 20.2$)] concentrations. AEA [RS = 0.5 ng/mL (95% CI= 0.09 to 0.8), ($d = 0.7$), ($P = 0.018$), (Fig. 3A)], 2-AG [RS = 0.04 ng/mL (95% CI= 0.01 to 0.06), ($d = 1.3$), ($P = 0.002$), (Fig. 3B)], dopamine [RS = 0.7 ng/mL (95% CI= 0.3 to 1), ($d = 1.1$), ($P = 0.001$), (Fig. 3D)], and β-endorphin [RS = 9.4 pg/mL (95% CI= 7 to 11.8), ($d = 2.2$), ($P<0.001$), (Fig. 3E)] concentrations significantly increased in the RS group but there were no significant changes in the RP group. However, serotonin concentrations [RS = 1.7 ng/mL (95% CI = 1.4 to 2.1), (d = 3), (P < 0.001) and RP = 1 ng/mL (95% CI, 0.5 to 1.5), (d = 1), (P = 0.001), (Figure 3C)] significantly increased in both groups with greater changes in RS group ($P = 0.021$). It should be noted that tryptophan concentrations remained unchanged in both groups [($P>0.05$), (Fig. 3F)].

3.2. Happiness levels

A significant group x time interaction was observed for happiness levels ($P < 0.001$). This variable significantly increased in both groups [RS = 1.5 number (95% CI= 1.2 to 1.8), ($d = 3$), ($P < 0.001$) and RP = 0.05 number (95% CI, 0.2 to 0.7), ($d = 0.9$), ($P = 0.003$), (Fig. 3G)] with greater changes in RS compared to RP group ($P < 0.001$).

3.3. Body composition

The body composition indices of the participants are presented in Table 2. A significant time effect was observed for BM [($P<0.001$), ($F = 18.5$)], BMI [($P<0.001$), ($F = 17.6$)], BFP [($P<0.001$), ($F = 41.5$)], and lean mass [($P<0.001$), ($F = 80.1$)]. BM [RS = 0.5 kg (95% CI= 0.2 to 0.7, ($d = 1$), ($P = 0.001$)] and BMI [RS = 0.1 kg.m^{-2} (95% CI= 0.05 to 0.2, ($d = 0.9$), ($P = 0.004$)] significantly ($P<0.05$) increased in RS group while BFP significantly decreased in the both groups [RS = −0.9% (95% CI= −1.1 to −0.6, ($d = 1.8$), ($P = 0.001$) RP = −0.3% (95% CI, −0.5 to −0.002), ($d = 0.6$), ($P = 0.048$)]. Moreover, lean mass significantly increased in both groups [RS = 0.8 kg (95% CI= 0.5 to 1 ($d = 1.7$), ($P < 0.001$) RP = 0.6 kg (95% CI, 0.4 to 0.8), ($d = 1.6$), ($P < 0.001$)]. No between-group significant difference was observed in any body composition variable.

3.4. Muscular endurance

The values of push-up are shown in Table 2. A significant group x time interaction was observed for Push-up [($P = 0.036$), ($F = 4.9$)], which was significantly increased in both groups [RS = 4.6 repetitions (95% CI= 3.9 to 5.3), ($d = 3.6$), ($P = 0.001$) and RP= 3.5 repetitions (95% CI, 2.8 to 4.3), ($d = 2.7$), ($P = 0.001$)] with greater changes in RS group ($P = 0.040$).

4. Discussion

This paper evaluated the effects of 6 weeks of saffron supplementation combined with RT on markers implicated in depression as well as happiness levels in untrained young males. The most striking result to emerge from our data is that 6 weeks of saffron supplementation combined with RT caused an improvement in happiness levels, serotonin, dopamine, β-endorphin, AEA, and 2-AG, but not tryptophan concentrations. These results are critically important as they expand on the health-promoting benefits of saffron and might, pending further research, unlock a potential therapy for improving happiness levels in RT populations [23].

Although there is an astonishing number of studies investigated the neuroprotective, anti-depressive, anti-inflammatory, and antioxidant effects of saffron supplementation, this is the first to evaluate the effects of RT combined with saffron supplementation on AEA, 2-AG, serotonin, dopamine, β-endorphin, and tryptophan concentrations as well as levels of happiness (via questionnaire) in untrained young males. Saffron has been used as an antidepressant and mood elevator in Persian traditional medicine to induce a sense of happiness and laughter [24]. Accumulated evidence demonstrates the effectiveness of saffron against depression in various animal models [25-27]. Hosseinzadeh et al. used forced swimming tests to show that saffron extracts [(aqueous (160–320 mg/kg) and ethanolic extracts (200–800 mg/kg)], as well as safranal and crocin, have antidepressant activities in mice [28]. These researchers stated that crocin and safranal decreased the immobility time and increased climbing and swimming times similar to imipramine and fluoxetine, respectively [28]. It is highly believed that neurotransmitters such as dopamine, norepinephrine, and serotonin have crucial roles in mood and depression [9]. With regard to the role of saffron as an antidepressant agent, research has reported that crocin might have an antagonistic action [9]. It has been suggested that safranal functions via serotonin and crocin might suppress the uptake of dopamine and norepinephrine [28,29]. Purushothuman et al. demonstrated that treatment with saffron (2 mL dissolved in water) saved dopaminergic cells in substantia nigra pars compacta (SNc) in mice [30]. Another study reported that crocin (25 mg/kg and 50 mg/kg) increased the expression of the cAMP response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) in the hippocampus of Wistar rats [31], suggesting that the antidepressant effects of crocin may be due to the increases of BDNF, CREB, and VEGF [31]. Crocin supplementation [(2 g of dried stigma extracted with 100 mL of methanol (80%)] has also been shown to influence serotonergic mechanisms through an antagonistic effect on the nonselective serotonin receptor (5-hydroxytryptamine), thus increasing serotonin uptake [32]. A significantly greater increment of serotonin in our RS group compared to RP highlights the beneficial effects of saffron supplementation.

The endocannabinoid system, including AEA and 2-AG, is located in both the central and peripheral nervous systems [33]. This system is responsible for numerous physiological tasks including sensations of pain [34] and mood disorders [35]. In addition, AEA has a preferential affinity for cannabinoid receptor 1 (CB_1), while 2-AG exhibits affinity and functional activity for both CB_1 and cannabinoid receptor 2 (CB_2) [36]. Studies regarding the effects of RT combined with saffron supplementation are lacking. However, there are some studies on physical activity in human and animal models. For instance, medium-intensity jogging resulted in an increased AEA concentration in healthy men and women compared to pre-training values [37]. Concentrations of AEA, but not 2-AG, also increased post-running in humans and dogs [38]. These results were confirmed in another study on healthy men after hiking [39]. Furthermore, AEA and 2-AG concentrations were elevated following 45 min of 70–75% $VO2_{max}$ in healthy adults [40]. The evidence for these results may have emerged from the hypothesis of increased BDNF and VEGF concentrations in neurogenesis resulting from endurance training [41]. In another study, an acute bout of RT has led to an increase in AEA and 2-AG in rats [42]. However, AEA, 2-AG, and β-endorphin concentrations remained unchanged after 8 weeks of RT in young men [7]. Based on limited studies, it seems that endurance training is better at increasing endocannabinoid system markers (AEA and 2-AG). With regard to the results that emerged from our study, it

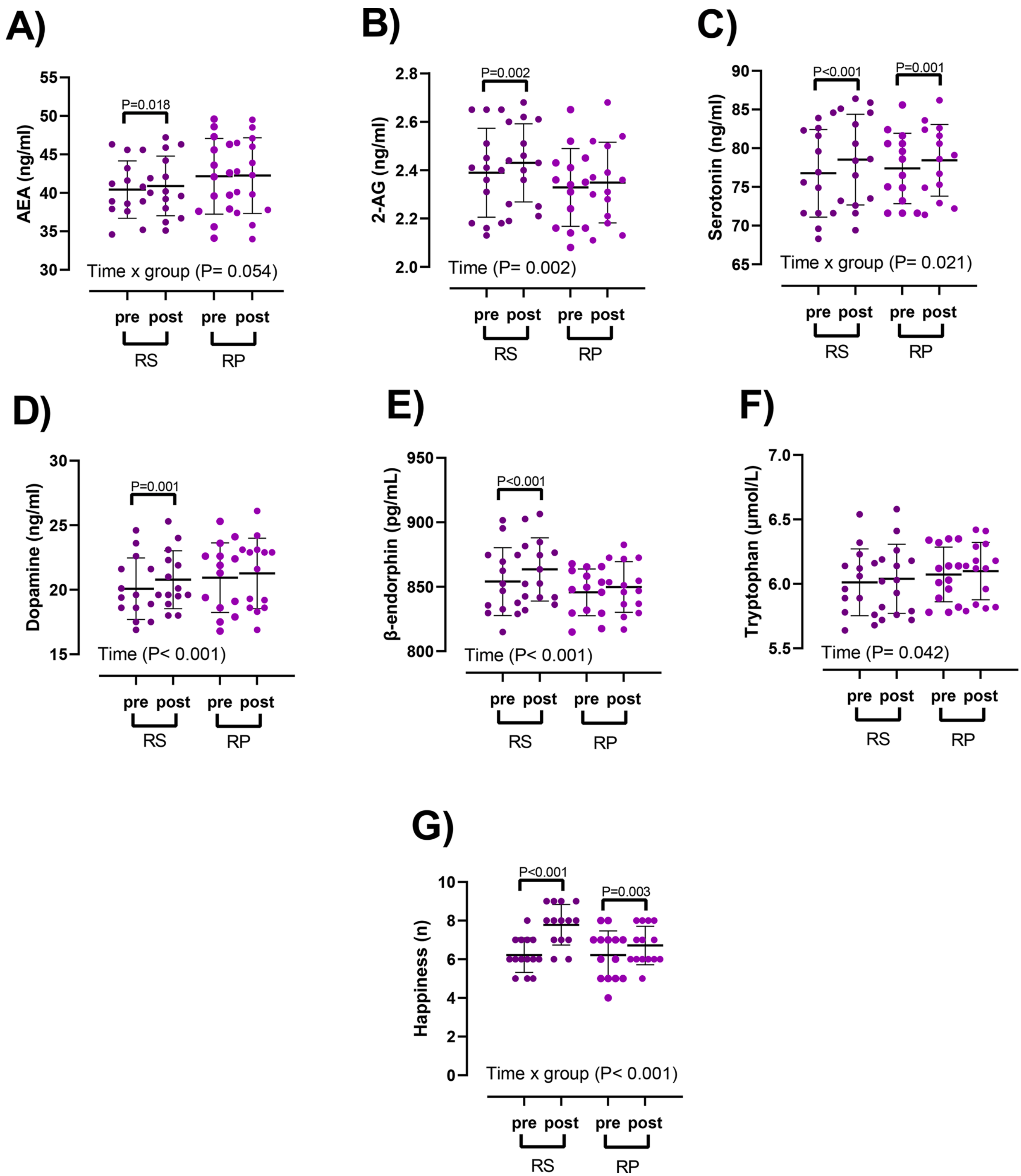

Fig. 3. Concentrations and levels of markers implicated in depression. **A)** Anandamide (AEA), **B)** 2-Arachidonoylglycerol (2-AG), **C)** Serotonin, **D)** Dopamine, **E)** β-endorphin, and **F)** Tryptophan, **G)** happiness. **Abbreviations**: ng/ml: nanogram/milliliter; pg/ml: picogram/milliliter; μmol/L: micromole/liter; n: number. *$p<0.05$ different from baseline.

Table 2
Physiological characteristics of participants.

Variables	Group	Pre-training	Post-training
BM (kg)	RS	77 ± 4.9	77.5 ± 4.8*
	RP	76.5 ± 3.9	76.7 ± 3.8
BMI (kg/m^2)	RS	25 ± 2.1	25.1 ± 2.1*
	RP	24.3 ± 1.5	24.3 ± 1.6
BFP (%)	RS	17.8 ± 1.5	16.9 ± 1.4*
	RP	17.4 ± 1.5	17.1 ± 1.7*
Lean mass (kg)	RS	59.1 ± 4.5	60 ± 4.6*
	RP	58 ± 2.4	58.6 ± 2.3*
Push-up (R)	RS	14.6 ± 4	19.2 ± 4.1*
	RP	14 ± 3.1	17.5 ± 3*

* $p<0.05$ different from baseline.Data are mean ± SD. Abbreviations: RS, resistance training+ saffron supplementation; RP, resistance training + placebo; BM, body mass; BFP, body fat percentage; BMI, body mass index; R, repetitions.

could be concluded that saffron had a surprising effect in increasing AEA and 2-AG concentrations due to its biological ingredients, such as crocin and safranal, which act as antidepressant agents.

In our study, happiness levels were increased in both groups with a greater increase in RS group. Moreover, we only observed a significant increase in β-endorphin concentrations in the RS group. Similar to our findings with the RP group, another study found no increase β-endorphin concentrations in young men after 8 weeks of RT [7]. In addition, β-endorphin concentrations remained unchanged after a single session of heavy RT in resistance-trained athletes [43]. However, various RT protocols significantly increased β-endorphin concentrations in healthy male participants [44]. Similarly, RT combined with both energy balance and energy restriction significantly increased β-endorphin in eumenorrheic females [45]. It appears that variations in β-endorphin concentrations in response to RT may be due to different protocols (various intensities, repetition range, rest intervals, etc.). In our study, β-endorphin concentrations only increased in the RS group, suggesting that saffron strengthened the β-endorphin release after RT.

Our results indicated that push-up endurance significantly increased in both groups with a greater increment in RS group. The influence of saffron on muscular strength and endurance adaptation to RT is understudied, as only one investigation reported beneficial effects of 10 days of saffron supplementation (300 mg/day) on maximum isometric and isotonic strength on a leg press machine in healthy male university students [46]. In view of the fact that the production of reactive oxygen species (ROS) during RT is promoted, the antioxidant qualities of saffron's ingredients may attenuate muscle fatigue [47], which seems to be a potential mechanism for increased muscular endurance. Further studies with a larger sample size and longer follow-up period are needed to evaluate the effects of saffron supplementation on muscular endurance or strength. Regarding body composition, we detected a significant decrement of BFP while an increase in lean mass in both groups after the training intervention with no between-group differences. The role of RT alone or combined with nutritional strategies on body composition in various populations has been vastly investigated and almost all of them reported an increase in lean mass and a decrement in fat mass or BFP [2, 48-51] through several mechanisms (e.g. myogenic pathways, hormones and cytokines, mechanical tension, muscle damage, metabolic stress, etc.) [52]. However, the role of saffron supplementation on body composition is very less studied. In our study, both groups significantly increased lean mass while decreased BFP with no significant change between groups, which suggests the insignificant role of saffron supplementation may be due to the lower protein content.

The following limitations should be taken into consideration when interpreting our results. We evaluated body composition via bioelectrical impedance, which is not as accurate as dual-energy X-ray absorptiometry (the gold standard technique for body composition measurements). However, previous studies have shown that it is a valid and reliable method [15,53]. Additionally, we were unable to measure the gene expression of our markers, which would have helped in explaining our results. Moreover, our participants were previously untrained young males. Therefore, the generalization of our findings to other cohorts should be avoided until further research is performed.

Another limitation of the current study is that participants had normal baseline happiness levels, producing a "ceiling effect" in which the improvement in happiness after our intervention might be less pronounced than those of populations with low happiness. This justifies further research in populations with decreased happiness to expand on our promising findings. It is also important to mention that although we used a reduced saffron dosage (150 mg/day) compared to other short-term studies [12,13], it is still considered a high dose that may not be recommended for certain individuals. Lastly, we used the push-up test (an upper-body activity) to evaluate muscular endurance. Since our RT program consisted of both upper and lower body exercises, the benefit of the RT intervention on muscular endurance might not be fully reflected by this specific test. Future studies should also aim at assessing lower body muscular endurance.

5. Conclusions

Saffron supplementation combined with RT improved the concentration of blood markers implicated in depression in untrained young males. Moreover, the addition of saffron supplement to chronic RT results in greater increases in levels of happiness than RT alone. A natural progression of this work is needed to evaluate the effects of different dosages of saffron supplementation in combination with RT in other populations, especially those with depression and low levels of happiness.

Conflict of interest

None of the authors reported a conflict of interest related to the study.

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