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ORIGINAL ARTICLE

The effects of saffron (*Crocus sativus L.*) in conjunction with concurrent training on body composition, glycaemic status, and inflammatory markers in obese men with type 2 diabetes mellitus: A randomized double-blind clinical trial

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Aims: Chronic inflammation is one of the major challenges in the management of obesity and type 2 diabetes mellitus (T2DM). Our primary aim was to assess the anti-inflammatory effects of Saffron (*Crocus sativus* L.) supplementation and concurrent training in obese men with T2DM.

Methods: Sixty obese men with T2DM (age = 39 ± 5 years; body mass = 93.9 ± 6 kg) were randomly assigned to four groups; concurrent training + placebo (CT; $n = 15$), saffron supplementation (S; $n = 15$), concurrent training + saffron supplementation (CTS; $n = 15$), or control (CON; $n = 15$). The participants in the CT group performed concurrent training (resistance + aerobic) three times per week for 12 weeks and received daily one pill of placebo (maltodextrin); the participants in the S group supplemented with one pill of 100 mg of saffron daily, and the participants in the CTS group participated in both saffron and training intervention while CON group continued regular lifestyle (no training and no supplementation). Inflammatory markers, body composition (evaluated by a multi-frequency bioelectrical impedance device; Jawon X-Contact 356), and metabolic profile were evaluated before and after interventions.

Results: All three interventions significantly ($P < .05$) decreased TNF-α (CT = −4.22, S = −1.91, CTS = −9.69 pg/mL), hs-CRP (CT = −0.13, S = −0.1, CTS = −0.32 ng/mL), IL-6 (CT = −6.84, S = −6.36, CTS = −13.55 pg/mL), IL-1β (CT = −8.85, S = −6.46, CTS = −19.8 pg/mL), FBG (CT = −6.97, S = −2.45, CTS = −13.86 mg/dL), insulin (CT = −0.13, S = −0.03, CTS = −0.21 mU/L), HOMA-IR (CT = −0.12, S = −0.04, CTS = −0.21), HbA1c (CT = −0.17, S = −0.11, CTS = −0.26%), and increased IL-10 (CT = 1.09, S = 0.53, CTS = 2.27 pg/mL) concentrations. There was a positive correlation between changes in BFP with hs-CRP, IL-6, IL-1β and TNF-α, and IL-10 concentrations across the intervention groups. Additionally, significant differences were observed between the changes for all variables in the CTS group compared to CT, S and CON groups ($P < .05$).

The authors confirm that the Principal Investigator for this paper is Babak Hooshmand Moghadam and that he had direct clinical responsibility for patients.The authors are not employed by the government of Iran and the individual authors are employed at an academic or research institution where research or education is the primary function of the entity and preparing articles in their "personal capacity" (in other words, "not as an official representative or otherwise on behalf of a sanctioned government").

Conclusion: It seems that an interaction of saffron supplementation and concurrent training has more efficient effects on anti-inflammatory status compared to saffron supplementation or concurrent training alone.

KEYWORDS
Crocus sativus, diabetes mellitus, inflammation, obesity, physical exercise training

1 | INTRODUCTION

Type 2 diabetes mellitus (T2DM) is the most prevalent metabolic disorder and the underlying cause of many complications related to morbidity and mortality.[1–3] This complex chronic disorder can impair the proper functioning of tissues and organs by activation of pathophysiological mechanisms such as oxidative stress, apoptosis, protein kinase c (PKC) isoforms, transcription factors and inflammation.[4,5] These events can lead to debilitating complications such as diabetic nephropathy, diabetic retinopathy, diabetic neuropathy, atherosclerosis, dementia and cardiovascular disease.[6]

Chronic inflammation plays a critical role in the pathogenesis and progression of diabetes and insulin resistance.[7–9] Production of inflammatory cytokines by adipose tissue may play a role in low-grade systemic inflammation.[5,10] It is well documented that excessive adipose tissue can lead to elevations of inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and high sensitivity C-reactive protein (hs-CRP) while reducing anti-inflammatory cytokines such as interleukin-10 (IL-10).[11–14] Further, these elevated inflammatory cytokines produced by adipocytes are linked with insulin resistance, inflammatory cascade and reduction in concentrations of insulin by β-cells and I-kappa B kinase β (NF-κB) pathway.[4] In light of this, there have been various studies showing the association of elevated inflammatory cytokines with T2DM, but results are still conflicting.[3,15–19] Further evidence suggests that modulating the inflammatory system can be of interest as a promising therapeutic strategy for T2DM.[4,7]

Nutritional and physical activity strategies have gained substantial interest in the prevention and treatment of non-communicable chronic diseases.[20–24] For instance, saffron (*Crocus sativus L.*) is a plant from the Iridaceae family and a carotenoid-rich spice. The chief bioactive components of saffron are crocin, crocetin, picrocrocin, and safranal, which could contribute to its wide range of biological properties.[23,25,26] Saffron has beneficial effects on modulating oxidative stress, inflammation, insulin resistance and high blood pressure.[20,26] This is partly due to the strong antioxidant and radical scavenging effects of its active components.[25] Promising anti-inflammatory effects of saffron supplementation have been observed in vitro and in vivo.[26,27] However, the effect of saffron supplementation on subclinical inflammation remains inconclusive. Several studies have shown that saffron supplementation may have a significant impact on concentrations of inflammatory biomarkers in different populations.[28–31] However, some other investigations have shown that saffron supplementation did not influence inflammatory biomarkers.[28–31]

Exercise training is another non-pharmaceutical strategy that can be integrated into therapy to reduce T2DM health complications.[2,32] Various studies indicate that aerobic training (AT) and resistance training (RT) separately improve systemic inflammation. Mechanistically, AT may improve inflammatory status through inhibition of monocyte

What is already known about this subject

- Evidence suggests that modulating the inflammatory system can be of interest as a promising therapeutic strategy for T2DM.
- Promising anti-inflammatory effects of saffron have been observed in vitro and in vivo. However, the effect of saffron on subclinical inflammation remains inconclusive, thereby limiting the dose and duration of use.
- Evidence indicated that aerobic training (AT) and resistance training (RT) separately improves systemic inflammation. However, studies in the field of concurrent training (RT + AT) that can be helpful in preventing and counteracting T2DM are very rare.

What this study adds

- Saffron supplementation improves inflammatory markers (TNF-α, hs-CRP, IL-6, IL-1β, IL-10), glycaemic markers (FPG, HbA1c, HOMA-IR, insulin), and body composition (BM, BMI, WHR, BFP) in obese men with TDM2.
- Concurrent training improves inflammatory markers (TNF-α, hs-CRP, IL-6, IL-1β, IL-10), glycaemic markers (FPG, HbA1c, HOMA-IR, insulin), and body composition (BM, BMI, WHR, BFP) in obese men with TDM2.
- Interaction of saffron supplementation and concurrent training have more efficient effects on anti-inflammatory status compared to saffron supplementation or concurrent training alone.

and macrophage infiltration into adipose tissue and the phenotypic switching of macrophages within adipose tissue.[33] However, RT may exert its anti-inflammatory actions through changes in body composition (reduction in body fat and increase/preserve lean body mass [LBM]).[34] Although, in theory, the combination of AT and RT might exert a stronger anti-inflammatory effect compared to AT or RT alone,[35] further research is required to understand the effects of concurrent training on inflammatory pathways in patients with T2DM.[36,37] To date, no studies have examined the effectiveness of saffron supplementation and concurrent training together on inflammatory and glycaemic markers in obese men with T2DM. Therefore, this study aimed to investigate the effects of 12 weeks of saffron supplementation and concurrent training on inflammatory markers (TNF-α, IL-6, IL-1β, hs-CRP and IL-10) in obese men with T2DM. It was hypothesized that saffron supplementation and concurrent training may provide additive benefits on inflammatory status.

2 | METHODS

2.1 | Participants

Sixty obese men with T2DM (age = 39 ± 5 years; body mass = 93.9 ± 6 kg) volunteered to take part in this study (Figure 1). Participants were recruited in Tehran, Iran. The inclusion criteria were 30–50 years, BMI > 30 kg/m^2, a diagnosis of T2M (defined according to the ADA criteria),[38] HbA1C ≥ 6.5% (48 mmol/mol), fasting blood glucose ≥ 126 mg/dL (7.0 mmol/L), and T2DM duration >2 years. Exclusion criteria were hepatic, renal, bone and cardiovascular diseases, severe hypertension, having chronic complications of diabetes (neuropathy and/or retinopathy), diabetes type 1, and taking medications (insulin, anticonvulsants, antihypertensive and lipid-lowering) except for oral hypoglycaemic agents (OHAs), namely metformin or glibenclamide, consuming nutritional supplements, consuming alcohol or smoking for at least 1 year before enrolling in the study, and having saffron allergy/sensitivity, and history of regular physical activity at least in the past year. All these criteria were evaluated by a physician using the Physical Activity Readiness-Questionnaire (PAR-Q) and medical health/history questionnaire. Written informed consent was obtained from all participants. All experimentation was carried out following the Declaration of Helsinki. The present study was approved by the Ethics Committee Ferdowsi University of Mashhad (IR.UM.REC.1399.009) and registered at the Iranian Registry of Clinical Trials (IRCT20190731044398N4).

2.2 | Study design

Before baseline measurements, all participants were familiarized with all testing and procedures. This study was a randomized, double-blind and parallel prospective clinical trial. Participants were randomly divided to four groups: concurrent training + placebo (CT; $n = 15$), saffron supplementation (S; $n = 15$), concurrent training + saffron supplementation (CTS; $n = 15$) or control (CON; $n = 15$). The allocation was stratified by using a digital tool available at www.randomizer.org. Participants in the CT group performed an exercise program three times a week for 12 weeks and received a placebo; participants in the S group took one pill containing 100 mg saffron daily, and the participants in the CTS group performed an exercise program and took saffron supplementation (one pill of 100 mg saffron). Participants in the CON group were asked to maintain a normal daily life pattern for the duration of the study. Measurement processes were collected at baseline and the end of 12 weeks of interventions (approximately 48 h after the last training session). All measurements were recorded at the same time of day (within ~1 h) and under the same environmental conditions (~20 °C and ~55% humidity). The participants were asked not to change their diet, medication and physical activity during the study period.

2.3 | Anthropometric assessments

Upon arriving at the laboratory, participants were asked to remain seated for 30 minutes prior to the test. Body mass (BM) was measured with a digital scale (SECA, Germany) to the nearest 0.1 kg. The participant's height was measured with a stadiometer (SECA, Germany) to the nearest 0.1 cm. In addition, to measure waist-to-hip ratio (WHR), the waist circumference was divided by the hip circumference. Body mass index (BMI), body fat percentage (BFP) and lean body mass (LBM) were evaluated by a multi-frequency bioelectrical impedance device (Jawon X-Contact 356, South Korea) as previously described.[39] The test–retest reliability of the bioelectrical impedance method is high ($R = 0.95$–0.99).

2.4 | Blood collection laboratory analysis

Fasting blood samples (~10 mL) were collected from the antecubital vein using standard procedures roughly 48 hours before and after the last training session. Following the completion of blood sampling, the samples were centrifuged at 1008 g-force for 10 minutes, and serum was stored at −80 °C until further analysis.

2.5 | Inflammatory markers

Serum TNF-α (kit: Cusabio Co., Houston, TX, USA, sensitivity: 1.59 pg/mL), hs-CRP (kit: Cusabio Co., Houston, TX, USA, sensitivity: 0.156 ng/mL), IL-1β (kit: Cusabio Co., Houston, TX, USA, sensitivity: 31.25 pg/mL), IL-6 (kit: Cusabio Co., Houston, TX, USA, sensitivity: 2.453 pg/mL) and IL-10 (kit: Cusabio Co., Houston, TX, USA, sensitivity: 3.12 pg/mL) concentrations were measured by using commercial human enzyme-linked immunosorbent assay (ELISA) kits. The intra- and inter-assay coefficients for all inflammatory factors were < 8% and < 10%, respectively.

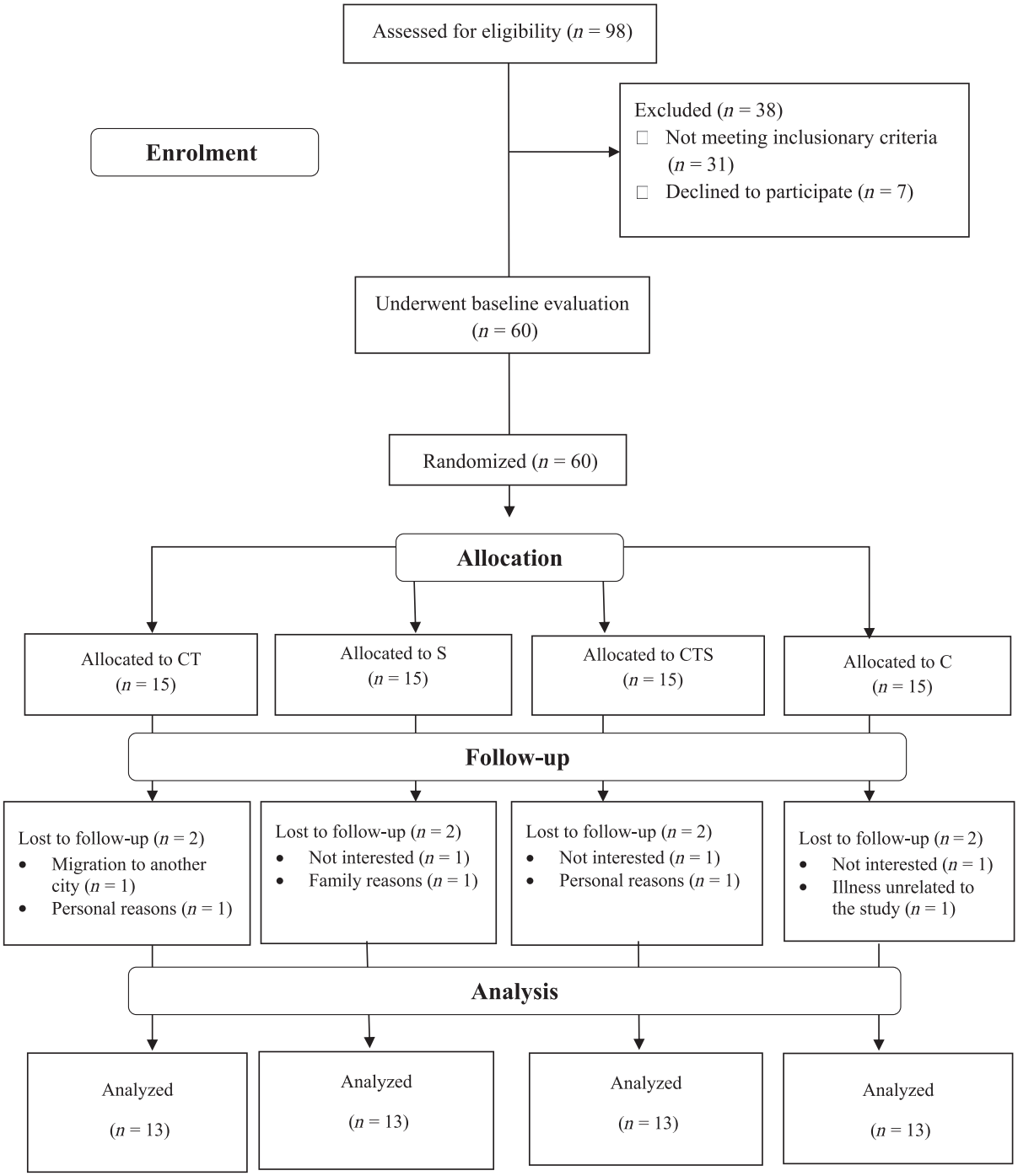

FIGURE 1 Participant flow diagram. CT, concurrent training + placebo; S, saffron; CTS, concurrent training + saffron; C, control

2.6 | Metabolic profile

HbA1c (immunoturbidimetry method, Pars Azmun, Iran), fasting plasma glucose (glucose oxidase method, Pars Azmun, Iran), and fasting insulin (kit: Cusabio Co., USA) concentrations were measured using commercial human kits. In addition, HOMA-IR (homeostasis model for insulin resistance) were calculated with fasting glucose (mg/dL) and fasting insulin (mU/L) values using the validated calculator (accessed at http://www.dtu.ox.ac.uk) (Score = (Fasting insulin) * (Fasting glucose)/405).[40]

2.6.1 | Concurrent training intervention

The training program was prescribed according to recommendations of the American College of Sports Medicine (ACSM) and

Americans with Disabilities Act (ADA) and following the current ACSM practical guidance for individuals with T2D.[41–43] To familiarize participants with the training procedures, those in the CT and CTS group were acquainted with the correct form of lifting and correct breathing techniques. To determine one repetition maximum (1RM), a warm-up was performed for 5–10 minutes. Subsequently, the relevant exercises were performed with minimum weights until fatigue. The participants performed two attempts and their heaviest successful lift and number of repetitions were recorded.[44] The number of repetitions to fatigue did not exceed ten. There was a 5 minute rest period between attempts. Finally, according to the following equation (Brzycki formula), 1RM was estimated for each exercise[45]: 1RM = weight/[1.0278 – (0.0278 × reps)].

In the CT and CTS group, participants performed the concurrent training program (circuit RT + AT) sessions on three non-consecutive days per week for 12 weeks. The training program consisted of four steps: a 10 minute warm-up, circuit RT, AT and a 10 minute cool-down. The training program was divided into four blocks. Each block comprised three-week microcycles (Table 1 shows details of the training program). RT (three sets/six exercises/60–90-second rest between each set/90–120-second rest between each exercise) included leg press, bench press, leg extension, lateral pulldown, lying leg curl and shoulder press.[43,46] The first training block began with 60–70% of 1RM) with 15–18 reps and progressed to 75–80% of 1RM with 8–10 reps at the end of the final training block (Table 1). The AT protocol was 10 × 1 minute high-intensity interval training on the treadmill at 80–95% of heart rate maximum (HR_{max}) interspersed with one-minute active rest at 40–60% of HR_{max}.[43] The first week began at 80% of HR_{max}, which was progressed to 95% HR_{max} in the last training block. Heart rate monitors (Polar T31, Oy, Kempele, Finland) were used to adjust workloads to achieve the target heart rate (Table 1). All training sessions were supervised by qualified personal trainers at a gym. The exercise orders were performed with one set of RT for all exercises (six exercises) and three sets of AT. Then, participants repeated this order in the second set of RT exercises and the next three sets of AT. Lastly, they repeated the third set of RT and the next last four sets of AT. The average duration of training was about 1–1.5 h/day.

2.6.2 | Saffron supplementation

Participants in the CTS group received one pill of 100 mg of pure saffron immediately after every training session and at the same time on non-training days for 12 weeks. Participants in the S group received one pill of 100 mg of pure saffron daily at the same times as the CTS group. In addition, participants in the CT group received daily the same amount of placebo (one pill contained 100 mg of maltodextrin). The appearance of the placebo pills (including colour and bundling) was similar to saffron supplement. The timing and dose of saffron were chosen according to the previous investigations.[26,47,48] The saffron pill (*C. sativus* L.) was supplied from Novin Saffron Company, Iran. The nutrient content of 1 g of saffron is approximately 19.7 mg of crocin and 0.25 mg of safranal. It should be noted that researchers administered the saffron supplementation after each training session. On non-training days, the supplementation was verified by a phone call or text message. To evaluate compliance with supplementation on non-training days, participants delivered the empty boxes of the supplement pills to the research staff.

2.6.3 | Nutrient intake and dietary analysis

Participants were asked to maintain their habitual diet during the study. To minimize dietary variability, participants submitted 3-day (2 weekdays and 1 weekend) food records at baseline and 12 weeks of the intervention. Each item of food was individually entered into Diet Analysis Plus version 10 (Cengage, Boston, MA, USA), and total energy consumption and the amount of energy derived from proteins, fats and carbohydrates were evaluated.[49]

2.6.4 | Statistical analysis

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 the concentration of inflammation markers following saffron supplementation in diabetic patients.[26,50] The analysis revealed a sample size of at least 52 participants ($n = 13$ per group) were needed to provide power

TABLE 1 Concurrent training intervention

			RT					AT	
Mesocycle	Week	Frequency (days/week)	Set	Repetition (*n*)	Rest between set (seconds)	Rest between exercise (seconds)	Intensity (1RM)	Intensity of one-minute activity)HR_{max})	Intensity of one-minute rest)HR_{max})
1	1–3	3	3	15–18	60–90	90–120	60–70	80	40–60
2	4–6	3	3	12–15	60–90	90–120	65–75	85	40–60
3	7–9	3	3	10–12	60–90	90–120	70–80	90	40–60
4	10–12	3	3	8–10	60–90	90–120	75–85	95	40–60

RT, resistance training; AT, aerobic training; 1RM, 1-repetition maximum; HR_{max}, heart rate maximum.

(1 − β) of 0.80 (α = 0.05). This number increased to 15 samples per group to cover the anticipated dropout. The normality of data was confirmed using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was used to examine possible group differences at baseline. Analysis of covariance (ANCOVA) was used to evaluate whether the means of variables/results pre- and post-test were equal across the sample. Pearson correlation was used to examine the relationship between BFP and inflammatory markers. All analyses and figures were performed with GraphPad Prism software (Version 8.4.3, GraphPad Software).

3 | RESULTS

3.1 | Dietary intake monitoring and compliance with exercise training and supplementation interventions

There were no reports of an adverse event from our saffron or training interventions. Nutrient analysis of the dietary records of the CT, S, CTS and CON groups before and after the intervention period is presented in Table 2. There were no significant changes in total energy intake and protein, fat and carbohydrate intakes between the study groups. Overall adherence to both exercise training and supplementation was 87% across CT, S and CTS.

3.1.1 | Inflammatory markers

Inflammatory markers are presented in Figure 2. All three interventions groups significantly ($P < .05$) decreased hs-CRP ([CT = −0.13 ng/mL; 95% CI, −0.16 to −0.09; $P < .001$], [CTS = −0.32 ng/mL; [95% CI, −0.41 to −0.22; $P < .0001$] and [S = −0.1 ng/mL; 95% CI, −0.13 to −0.07; $P < .001$]) (Figure 2A); IL-6 ([CT = −6.84 pg/mL; 95% CI, −9.35 to −4.33; $P < .001$], [CTS = −13.55 pg/mL; 95% CI, −16.54 to −10.56; $P < .001$] and [S = −6.36 pg/mL; 95% CI, −8.63 to −4.08; $P < .001$]), (Figure 2B); IL-1β ([CT = −8.85 pg/mL; 95% CI, −10.47 to −7.3; $P < .001$], [CTS = −19.80 pg/mL; 95% CI, −24.42 to −15.17; $P < .001$] and [S = −6.46 pg/mL; 95% CI, −8.16 to −4.77; $P < .001$]), (Figure 2C); TNF-α ([CT = −4.22 pg/mL; 95% CI, −6.04 to −2.40; $P < .001$], [CTS = −9.69 pg/mL; 95% CI, −12.05 to −7.32; $P < .001$] and [S = −1.91 pg/mL; 95% CI, −3.36 to −0.45; $P = .014$]), (Figure 2D), while significantly ($P < .05$) increased IL-10 ([CT = 1.09 pg/mL; 95% CI, 0.64 to 1.53; $P < .001$], [CTS = 2.27 pg/mL; 95% CI, 1.55 to 3.00; $P < .001$] and [S = 0.53 pg/mL; 95% CI, 0.17 to 0.89; $P < .001$]), (Figure 2E) concentrations over time. There were not any alterations in these variables in the CON group ($P > .05$). Post-test of all concentrations of inflammatory markers were significantly greater in CTS compared to all other groups (Table 3). There was a positive correlation between changes in BFP with hs-CRP ($r = 0.42$, $P = .002$), IL-6 ($r = 0.49$, $P < .001$), IL-1β ($r = 0.46$, $P = .001$), TNF-α ($r = 0.42$, $P = .002$) and IL-10 ($r = 0.47$, $P < .001$) concentrations across the intervention groups.

3.1.2 | Metabolic profile

Metabolic profile data are presented in Figure 3. All three intervention groups (CT, CTS and S) showed significant reductions in FPG ([CT = −6.97 mg/dL; 95% CI, −9.06 to −4.88; $P < .001$], [CTS = −13.86 mg/dL; 95% CI, −15.70 to −12.01; $P < .001$] and

TABLE 2 Energy and macronutrients at baseline and at the end of 12 weeks

Variables	Group	Pre-test	Post-test	*P*-value
Energy intake (kcal/day)	CON	1949.07 ± 52.34	1935.07 ± 63.93	0.315
	CT	1966.76 ± 57.29	1946.30 ± 39.59	0.130
	CTS	1958.23 ± 45.90	1965.92 ± 58.48	0.670
	S	1958.38 ± 65.33	1942.92 ± 47.73	0.352
Carbohydrate (g/day)	CON	270.30 ± 7.28	268.84 ± 10.70	0.508
	CT	271.61 ± 7.57	269.46 ± 7.28	0.245
	CTS	267.23 ± 8.35	271.76 ± 10.19	0.156
	S	270.53 ± 7.44	267.46 ± 9.48	0.330
Protein (g/day)	CON	80.92 ± 6.04	78.84 ± 6.05	0.287
	CT	80.92 ± 5.17	81.76 ± 5.67	0.579
	CTS	83.00 ± 7.03	81.76 ± 4.04	0.367
	S	79.38 ± 5.62	79.46 ± 4.94	0.497
Fat (g/day)	CON	60.46 ± 3.17	59.00 ± 4.43	0.129
	CT	61.84 ± 3.43	60.15 ± 2.44	0.217
	CTS	61.92 ± 2.95	61.30 ± 3.54	0.605
	S	62.07 ± 4.31	61.69 ± 4.06	0.748

All data are presented as mean ± SD. CON, control group; CT, concurrent training + placebo group; CTS, concurrent training + saffron group; S, saffron group. Significant data are set at $P < .05$.

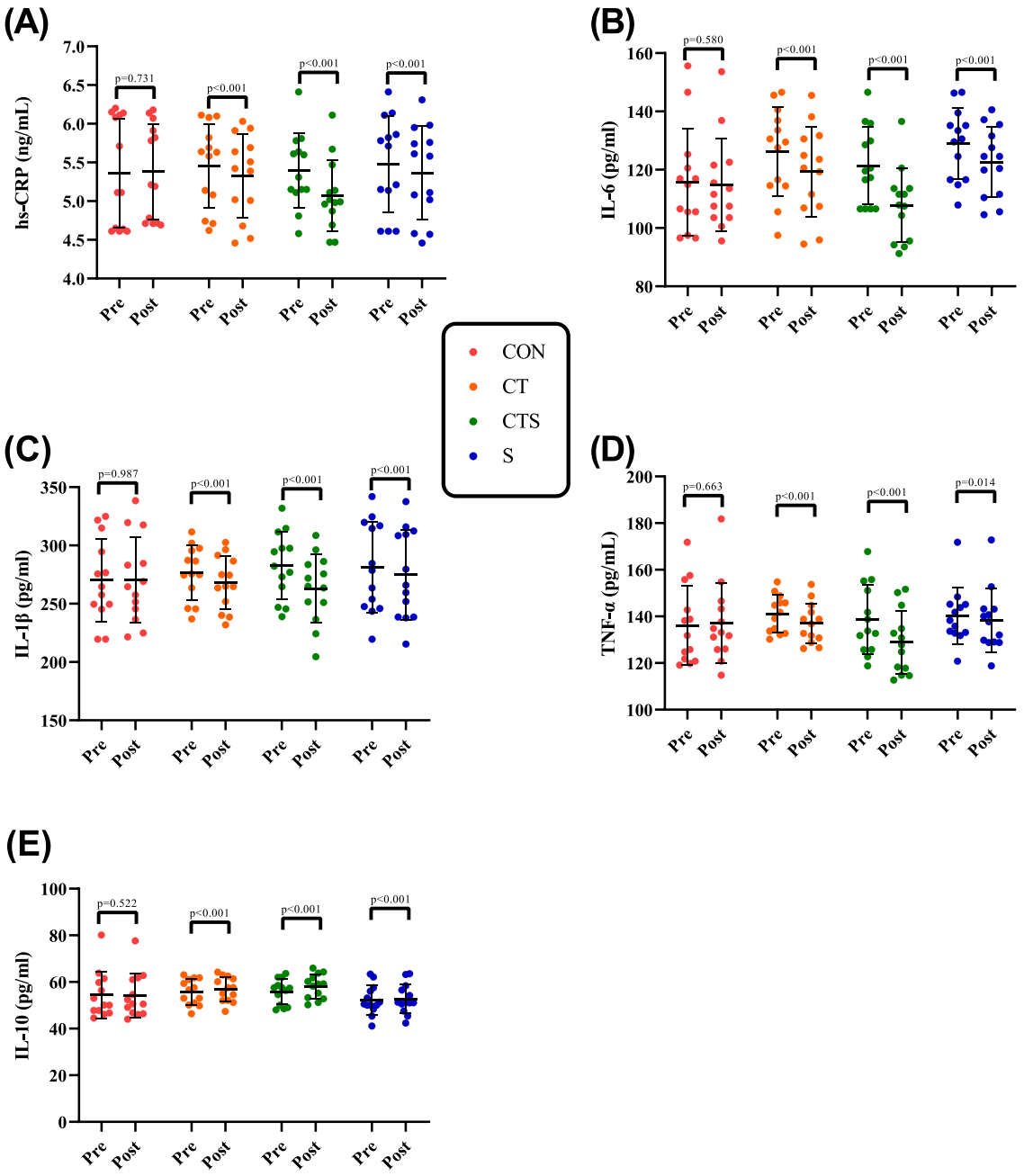

FIGURE 2 Inflammatory blood markers in response to 12 weeks of saffron and concurrent training. CON, control; CT, concurrent training + placebo; CTS, concurrent training + saffron; S, saffron. (A) hs-CRP, high sensitivity reactive protein; (B) IL-6, interleukin-6; (C) IL-1β, interleukin-1β; (D) TNF-α, tumour necrosis factor-α; (E) IL-10, interleukin-10. Error bars indicate SD

[S = − 2.45 mg/dL; 95% CI, −3.45 to −1.45; $P < .001$]), (Figure 3A); HOMA-IR ([CT = −0.12; 95% CI, −0.14 to −0.09; $P < .001$], [CTS = −0.21; 95% CI, −0.24 to −0.18; $P < .001$] and [S = − 0.04; 95% CI, −0.05 to −0.02; $P < .001$]), (Figure 3B) and HbA1c ([CT = −0.17%; 95% CI, −0.21 to −0.13; $P < .001$], [CTS = −0.26%; 95% CI, −0.33 to −0.19; $P < .001$] and [S = − 0.11%;, 95% CI, −0.13 to −0.08; $P < .001$]), (Figure 3C) over time. In addition, results showed a significant reduction in insulin concentrations in three intervention groups ([CT, −0.13 mU/L; 95% CI, −0.17 to −0.09; $P < .001$], [CTS = −0.21 mU/L; 95% CI, −0.25 to −0.17; $P < .001$] and [S = −0.03 mU/L; 95% CI, −0.008 to 0.06; $P = .013$]), (Figure 3D)] over time, while no significant difference was found in the CON group ($P > .05$). Post-test of all metabolic data were significantly greater in CTS compared to all other groups (Table 4).

3.1.3 | Body composition

Anthropometric characteristics of participants are shown in Figure 4. Three intervention groups (CT, CTS and S) showed significant

TABLE 3 The impact of group on the post values controlling pre values using ANCOVA and Bonferroni as multiple comparison test

Dependent variable	Contrast	β (SE)	95% CI	*P*-value
hs-CRP-post	CT *vs.* CTS	0.19 (0.04)	0.07 to 0.3	<0.001
	CT *vs.* CON	−0.13 (0.04)	−0.25 to −0.02	0.010
	CT *vs.* S	−0.02 (0.04)	−0.14 to 0.08	1.000
	CTS *vs.* CON	−0.33 (0.04)	−0.44 to −0.21	<0.001
	CTS *vs.* S	−0.21 (0.04)	−0.33 to −0.1	<0.001
	CON *vs.* S	0.11 (0.04)	0.00 to 0.22	0.053
IL-6-post	CT *vs.* CTS	7.2 (1.75)	2.35 to 12.04	0.001
	CT *vs.* CON	−4.88 (1.80)	−9.85 to 0.07	0.056
	CT *vs.* S	−0.78 (1.75)	−5.6 to 4.04	1.000
	CTS *vs.* CON	−12.08 (1.76)	−16.94 to −7.22	<0.001
	CTS *vs.* S	−7.98 (1.77)	−12.87 to −3.08	<0.001
	CON *vs.* S	4.1 (1.83)	−0.95 to 9.16	0.181
IL-1β-post	CT *vs.* CTS	10.9(2.36)	4.4 to 17.41	<0.001
	CT *vs.* CON	−8.84 (2.36)	−15.35 to −2.34	0.003
	CT *vs.* S	−2.41 (2.35)	−8.91 to 4.08	1.000
	CTS *vs.* CON	−19.75 (2.38)	−26.31 to −13.19	<0.001
	CTS *vs.* S	−13.32 (2.35)	−19.81 to −6.82	<0.001
	CON *vs.* S	6.43 (2.37)	−0.1 to 12.97	0.056
TNF-α-post	CT *vs.* CTS	5.61 (1.95)	0.23 to 10.98	0.036
	CT *vs.* CON	−4.96 (1.96)	−10.37 to 0.44	0.089
	CT *vs.* S	−2.25 (1.94)	−7.61 to 3.1	1.000
	CTS *vs.* CON	−10.57 (1.95)	−15.94 to −5.2	<0.001
	CTS *vs.* S	−7.86 (1.94)	−13.22 to −2.50	0.001
	CON *vs.* S	2.70 (1.95)	−2.68 to 8.1	1.000
IL-10-post	CT *vs.* CTS	−1.19 (0.41)	−2.33 to −0.04	0.037
	CT *vs.* CON	1.44 (0.41)	0.29 to 2.58	0.007
	CT *vs.* S	0.71 (0.42)	−0.44 to 1.87	0.564
	CTS *vs.* CON	2.63 (0.41)	1.48 to 3.77	<0.001
	CTS *vs.* S	1.90 (0.42)	0.74 to 3.07	<0.001
	CON *vs.* S	−0.72 (0.41)	−1.86 to 0.42	0.543

hs-CRP, high sensitivity reactive protein; TNF-α, tumour necrosis factor-α; IL-6, interleukin-6; IL-1β, interleukin-1β; IL-10, interleukin-10; FM, fat mass; WHR, waist hip ratio; BFP, body fat percent; LBM, lean body mass; CON, control group; CT, concurrent training + placebo group; CTS, concurrent training + saffron group; S, saffron group.

reductions in BM ([CT = −1.53 kg; 95% CI, −1.91 to −1.15; $P < .001$], [CTS = −2.89 kg; 95% CI, −3.33 to −2.46; $P < .001$] and [S = −0.89 kg; 95% CI, −1.18 to −0.59; $P < .001$]), (Figure 4A), BMI ([CT = −0.5 kg/m^2; 95% CI, −0.64 to −0.38; $P < .001$], [CTS = −0.9 kg/m^2; 95% CI, −0.84 to −1; $P < .001$] and [S = −0.2 kg/m^2; 95% CI, −0.19 to −0.39; $P < .001$]), (Figure 4B), WHR ([CT = −0.04 cm; 95% CI, −0.04 to −0.03; $P < .001$], [CTS = −0.06 cm; 95% CI, −0.08 to −0.04; $P < .001$] and [S = −0.02 cm; 95% CI, −0.02 to −0.00; $P = .001$]), (Figure 4C), BFP ([CT = −0.6%; 95% CI, −0.29 to −0.96; $P = .001$], [CTS = −1.7%; 95% CI, −1.13 to −2.27; $P < .001$] and [S = 0.04%; 95% CI, −0.2 to −0.67; $P = .002$]), (Figure 4D) over time. There were no significant changes in these variables in the CON group ($P > .05$). In regards to LBM, there was only a significant reduction ([CT = −0.39 kg; 95% CI, −0.68 to −0.08; $P = .016$]), (Figure 4E) in the CT group. Post-test of all body composition data except for LBM were significantly greater in CTS compared to all other groups (Table 5).

4 | DISCUSSION

In the present study, we examined the effects of saffron supplementation and concurrent training in a circuit manner on body composition, inflammatory and glycaemic markers in obese men with T2DM. To the best of our knowledge, this is the first study to investigate the anti-inflammatory effects of saffron and concurrent training in obese men with T2DM. The results of the present study showed that all three interventions (CT, S and CTS) significantly decreased BM, BMI,

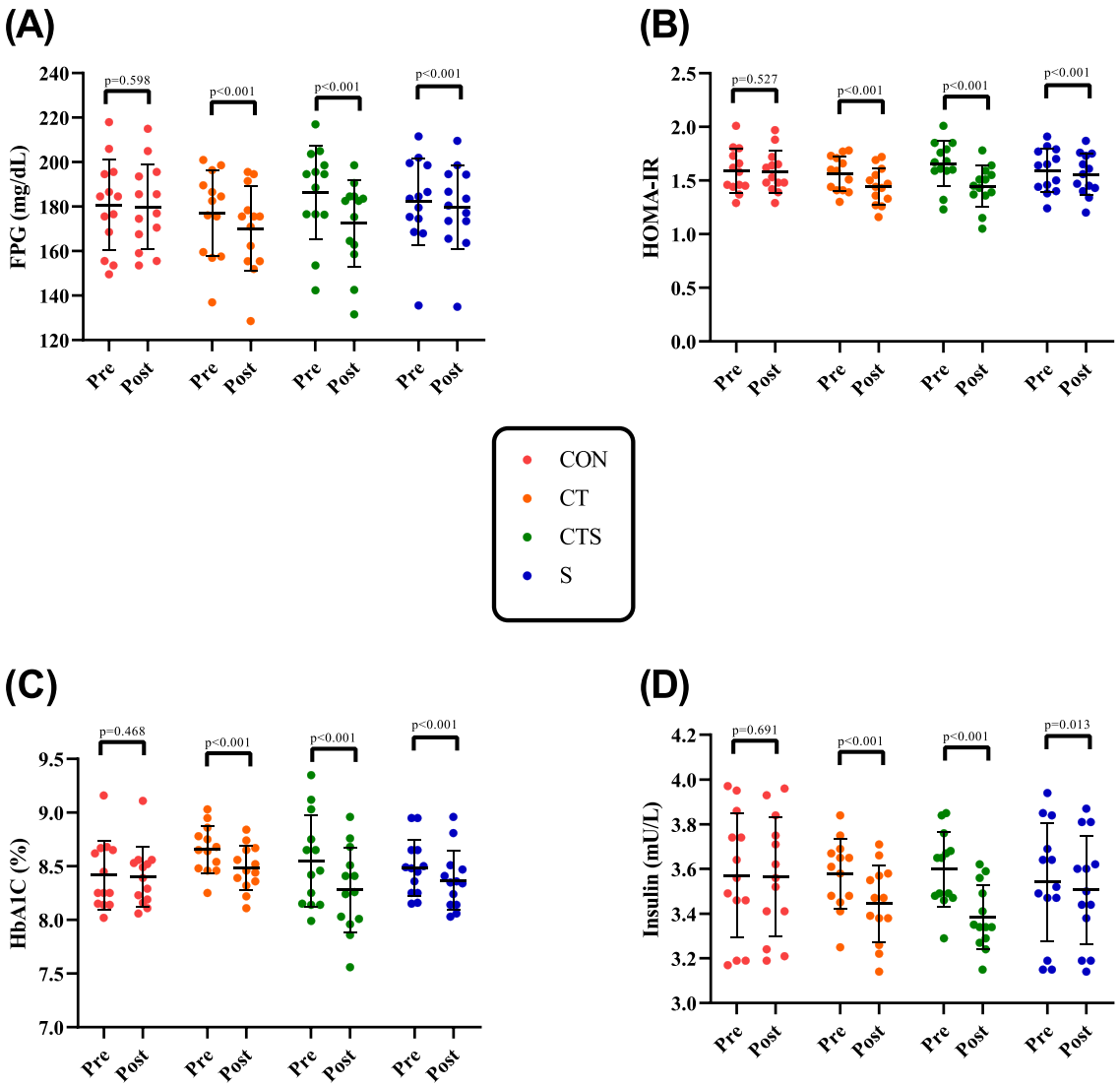

FIGURE 3 Metabolic profile in response to 12 weeks of saffron and concurrent training. CON, control; CT, concurrent training + placebo; CTS, concurrent training + saffron; S, saffron; FPG, fasting plasma glucose. Error bars indicate SD

WHR, BFP, FPG, HbA1c, HOMA-IR, insulin, TNF-α, hs-CRP, IL-6, IL-1β, while increased IL-10 concentrations. Post-test changes in these variables were significantly greater in the CTS group compared to the other groups. Also, there was a positive correlation between changes in BFP with hs-CRP, IL-6, IL-1β, TNF-α and IL-10 concentrations across the intervention groups.

Saffron supplementation significantly lowered FPG, HbA1c, HOMA-IR and insulin. It has been found that saffron supplementation improves HbA1c by neutralizing free radicals and increasing the activity of antioxidant enzymes.[51] In this regard, Kermani et al. have reported that 100 mg of crocus sativus supplementation for 12 weeks significantly decreased FPG in subjects with metabolic syndrome[50] while Ebrahimi et al. found no significant reduction in HbA1c, FPG and serum insulin concentrations following 12 weeks of 100 mg saffron supplementation in T2DM patients.[48] In the present study, we showed reduced FGP and HbA1c concentrations in CTS, S and CT groups. There are some potential mechanisms by which saffron supplementation might have provided beneficial effects on glycaemic markers, including inhibition of renal glucose reabsorption, reduction of insulin resistance by stimulating and regenerating of β-cells in islets of Langerhans, enhancement of glucose uptake, and insulin sensitivity in skeletal muscle cells mediated by adenosine monophosphate-activated protein kinase/acetyl-CoA carboxylase (AMPK/ACC), and mitogen-activated protein kinases (MAPKs) pathways.[52,53] It has been suggested that the principal mechanisms involved in the antidiabetic effect of saffron supplementation are its strong antioxidant and anti-inflammatory properties.[54] This evidence implies that the active compounds of saffron supplementation can alter molecular mechanisms by affecting transcription factors, growth factors and diverse intracellular signalling pathways. Improvement of the glycaemic profile by saffron components can prevent diabetic complications by inhibition of hyperglycaemia-induced pathophysiologic molecular pathways.[55] On the other hand, it has been demonstrated that exercise training combined with dietary modification was linked to lower HbA1c

TABLE 4 The impact of group on the post values controlling pre values using ANCOVA and Bonferroni as multiple comparison test

Dependent variable	Contrast	β (SE)	95% CI	*P*-value
FPG-post	CT *vs.* CTS	6.32 (1.43)	2.36 to 10.27	<0.001
	CT *vs.* CON	−6.33 (1.42)	−10.24 to −2.42	<0.001
	CT *vs.* S	−4.83 (1.42)	−8.75 to −0.91	0.008
	CTS *vs.* CON	−12.65 (1.42)	−16.58 to −8.73	<0.001
	CTS *vs.* S	−11.15 (1.42)	−15.06 to −7.23	<0.001
	CON *vs.* S	1.50 (1.41)	−2.39 to 5.41	1.000
Insulin-post	CT *vs.* CTS	0.8 (0.02)	0.02 to 0.13	0.003
	CT *vs.* CON	−0.12 (0.02)	−0.18 to −0.06	<0.001
	CT *vs.* S	−0.09 (0.02)	−0.15 to −0.03	<0.001
	CTS *vs.* CON	−0.2 (0.02)	−0.26 to −0.14	<0.001
	CTS *vs.* S	−0.17 (0.02)	−0.23 to −0.11	<0.001
	CON *vs.* S	0.03 (0.02)	−0.02 to −0.09	0.857
HOMA-IR-post	CT *vs.* CTS	0.09 (0.01)	0.04 to 0.13	<0.001
	CT *vs.* CON	−0.1 (0.01)	−0.15 to −0.06	<0.001
	CT *vs.* S	−0.08 (0.01)	−0.12 to −0.03	<0.001
	CTS *vs.* CON	−0.19 (0.01)	−0.24 to −0.15	<0.001
	CTS *vs.* S	−0.17 (0.01)	−0.21 to −0.12	<0.001
	CON *vs.* S	0.02 (0.01)	−0.01 to −0.07	0.580
HbA1c-post	CT *vs.* CTS	0.1 (0.02)	0.02 to 0.18	0.006
	CT *vs.* CON	−0.13 (0.03)	−0.21 to −0.05	<0.001
	CT *vs.* S	−0.04 (0.03)	−0.12 to 0.03	0.833
	CTS *vs.* CON	−0.23 (0.02)	−0.31 to −0.15	<0.001
	CTS *vs.* S	−0.14 (0.02)	−0.22 to −0.06	<0.001
	CON *vs.* S	0.08 (0.02)	0.009 to 0.16	0.023

FPG, fasting plasma glucose; CON, control group; CT, concurrent training + placebo group; CTS, concurrent training + saffron group; S, saffron group.

concentration in patients with T2DM.[56] Combined AT and RT could be an effective strategy in reducing blood lipids and improving glycaemic regulation in patients with T2DM.[56] In agreement with our results, Tomas-Carus et al. have demonstrated that 60 minutes of combined AT and RT for 12-weeks significantly decreased HbA1C concentration in T2DM patients.[57] In addition, results of the present study revealed significant differences in concentrations of FPG, insulin and HOMA-IR in CT compared to S. It seems that concurrent training increases glucose uptake, which is the main factor in the improvement of FPG, insulin, HOMA-IR and HbA1C concentrations through increased glucose uptake capacity, muscular blood flow and glucose transporter type 4 (GLUT4). These findings indicate that there may be an additive effect of saffron supplementation and concurrent training in improving glycaemic markers in obese men with T2DM.

Elevated inflammatory cytokines are the most accepted theories in mediating T2DM development.[58,59] Pro-inflammatory cytokines such as TNF-α, IL-6, hs-CRP and IL-1β are the main markers, which can regulate the expression of other inflammatory factors, and enhancement of these cytokines increase the prevalence of T2DM. The elevated level of cytokines is reported to result in the dysfunction of β-cell, which has been identified as a key component in the development of insulin resistance and diabetes mellitus progression.[31] The probable effects of saffron supplementation on pro- and anti-inflammatory cytokines are contradictory. For instance, Rajabi et al. indicated that 8 weeks of AT combined with 400 mg of saffron supplementation daily significantly reduced IL-6 and TNF-α concentrations in T2D women.[60] In a recent study, Tajik et al. indicated that 12 weeks of RT combined with 40 mg of saffron supplementation daily significantly reduced hs-CRP concentrations.[61] In another study, 8 weeks of saffron supplementation daily in patients with T2D significantly diminished glucose as well as the expression of some inflammatory markers such as TNF-α and IL-6.[26] Faridi et al. evaluated the effects of 3 weeks of hydroalcoholic extract of saffron in diabetic mice. They reported that 500 mg/kg of saffron supplementation daily significantly increased IL-10 expression.[62] Conversely, Ebrahimi et al. reported no notable changes in TNF-α and FPG concentrations in patients with T2DM who supplemented by taking 100 mg of saffron for 12 weeks.[48] It was found that 100 mg crocetin could significantly reduce IL-1 β, IL-6 and TNF-α concentrations in diabetic rats.[31] Furthermore, it has been reported that crocetin can decrease TNF-α, IL-6, hs-CRP and IL-1β concentrations and also increases the production of IL-10.[63] A review study concerning lethal dose (LD50) of saffron reported that although ingestion of less than 1.5 g of saffron is not toxic for humans, doses

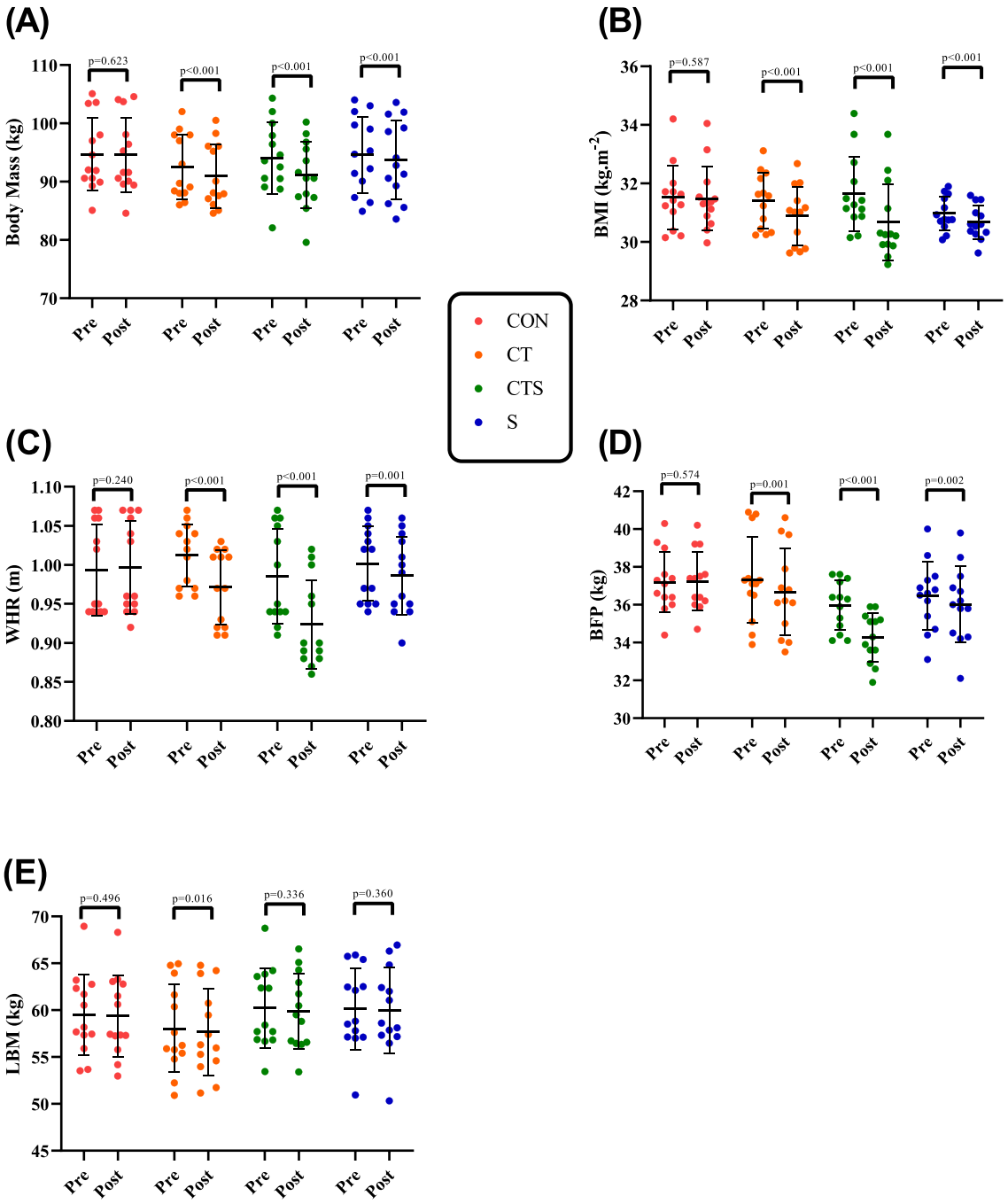

FIGURE 4 Body composition characteristic in response to 12 weeks of saffron and concurrent training. CON, control; CT, concurrent training + placebo; CTS, concurrent training + saffron; S, saffron; BM, body mass; BMI, body mass index; WHR, waist hip ratio; BFP, body fat percent; LBM, lean body mass. Error bars indicate SD

higher than 5 g/day will be considered toxic.[64] These disagreements in the literature may be due to different doses, different metabolite content of the administered supplements, intervention duration and characteristics of participants.

The exact mechanism explaining the effect of saffron supplementation on anthropometric and inflammatory biomarkers is still unknown. However, it was proposed that the anti-inflammatory properties of saffron are due to its potent antioxidant and radical scavenging function.[51,53] Saffron contains more than 150 chemical compounds including vitamins (such as thiamine and riboflavin), amino acids (alanine, proline and aspartic acid), polysaccharides, flavonoids and carotenoids (including crocin and crocetin), which are known as key regulators of the antioxidant and inflammatory responses.[20] The antioxidant properties of crocin reduce the levels of endogenously generated reactive oxygen species, particularly hydrogen peroxide, in platelets, and consequently, inhibit oxidative stress, which plays an

TABLE 5 The impact of group on the post values controlling pre values using ANCOVA and Bonferroni as multiple comparison test

Dependent variable	Contrast	β (SE)	95% CI	*P*-value
BM-post	CT *vs.* CTS	1.34 (0.25)	0.62 to 2.05	<0.001
	CT *vs.* CON	−1.44 (0.26)	−2.15 to −0.72	<0.001
	CT *vs.* S	−0.65 (0.26)	−1.37 to 0.06	0.092
	CTS *vs.* CON	−2.78 (0.25)	−3.49 to −2.07	<0.001
	CTS *vs.* S	−1.99 (0.25)	−2.7 to −1.28	<0.001
	CON *vs.* S	−0.78 (0.25)	−1.49 to −0.07	0.023
BMI-post	CT *vs.* CTS	0.45 (0.08)	0.21 to 0.68	<0.001
	CT *vs.* CON	−0.47 (0.08)	−0.7 to −0.24	<0.001
	CT *vs.* S	−0.22 (0.08)	−0.45 to 0.01	0.073
	CTS *vs.* CON	−0.92 (0.08)	−1.15 to −0.69	<0.001
	CTS *vs.* S	−0.67 (0.08)	−0.9 to −0.43	<0.001
	CON *vs.* S	0.25 (0.08)	0.02 to 0.49	0.025
WHR-post	CT *vs.* CTS	0.02 (0.008)	0.001 to 0.04	0.039
	CT *vs.* CON	−0.04 (0.008)	−0.06 to −0.02	<0.001
	CT *vs.* S	−0.02 (0.008)	−0.46 to −0.004	0.011
	CTS *vs.* CON	−0.06 (0.008)	−0.08 to −0.04	<0.001
	CTS *vs.* S	−0.04 (0.008)	−0.06 to −0.02	<0.001
	CON *vs.* S	0.01 (0.008)	−0.002 to 0.04	0.094
BFP-post	CT *vs.* CTS	1.11 (0.24)	0.44 to 1.78	<0.001
	CT *vs.* CON	−0.66 (0.23)	−1.31 to −0.02	0.039
	CT *vs.* S	−0.17 (0.23)	−0.82 to 0.48	1.000
	CTS *vs.* CON	−1.77 (0.24)	−2.44 to −1.11	<0.001
	CTS *vs.* S	−1.28 (0.23)	−1.93 to −0.63	<0.001
	CON *vs.* S	−0.49 (0.23)	−1.14 to 0.15	0.251
LBM-post	CT *vs.* CTS	−0.12 (0.28)	−0.91 to 0.66	1.000
	CT *vs.* CON	−0.3 (0.28)	−1.08 to 0.47	1.000
	CT *vs.* S	−0.26 (0.28)	−1.05 to 0.52	1.000
	CTS *vs.* CON	−0.17 (0.28)	−0.95 to 0.6	1.000
	CTS *vs.* S	−0.14 (0.28)	−0.92 to 0.63	1.000
	CON *vs.* S	−0.03 (0.28)	−0.81 to 0.74	1.000

BM, body mass; BMI, body mass index; WHR, waist hip ratio; BFP, body fat percent; LBM, lean body mass; CON, control group; CT, concurrent training + placebo group; CTS, concurrent training + saffron group; S, saffron group.

important role in the production of pro-inflammatory biomarkers.[23] In an experimental study, western blot results showed that the protein expression of the phosphatidylinositol 3-kinase (PI3K) and p-Akt were significantly improved by crocin supplementation in diabetic rats.[31] PI3K/Akt signalling plays a significant role in oxidative stress blockage and suppression of pro-inflammatory responses to oxidative stress.[31] In addition, crocin prevents inflammatory-induced damage, glycation of serum proteins, and improves insulin sensitivity in skeletal muscle through the crosstalk with AMP-activated protein kinase (AMPK). The activation of AMPK promotes GLUT4, which leads to the improvement of insulin-stimulated glucose uptake.[51]

Physical activity is considered a beneficial strategy with anti-inflammatory properties, which reduces the risk of inflammatory-related diseases.[1] The concomitant integration of RT and AT within a periodized training program is termed 'concurrent training' or circuit training.[65,66] Given the capacity of both exercise modes to induce adaptations within the skeletal muscle that counteract several disorders impacting upon functional capacity and metabolic health, including type II diabetes and obesity, concurrent training appears to be an attractive exercise strategy for preventing and counteracting multiple disease states.[65] In a study, Salamat et al. demonstrated that IL-6 concentrations were significantly decreased after 8 weeks of AT, RT and concurrent training, whereas this was not observed in the TNF-α and IL-1β concentrations in overweight men.[67] Nikseresht et al. reported that IL-10, IL-20 and TNF-α concentrations did not significantly change following 12 weeks of nonlinear RT and aerobic interval training in sedentary obese men.[68] In addition, Touvra et al. found a significant reduction in hs-CRP concentrations without any observed alterations in IL-6, IL-10 and TNF-α concentrations in patients with T2DM who

participated in an 8-week combined RT and AT program.[69] In addition, Di Blasio et al. showed alternating endurance resistance training in a circuit manner can be a useful tool due to the higher increase from baseline of fat-powered energy consumption compared to other exercise protocols (endurance-resistance training and resistance-endurance training).[34] Some mechanisms may be involved in the anti-inflammatory effects of exercise in TDM2 patients. AT may improve inflammatory status through inhibition of monocyte and macrophage infiltration into adipose tissue and the phenotypic switching of macrophages within adipose tissue,[33] while RT may reduce body fat, increase or preserve LBM and decrease inflammation.[70,71] However, a combination of AT and RT may exert a stronger anti-inflammatory effect compared to AT or RT alone.[35] Previous studies indicated that regular exercise training significantly reduced oxidative stress by the increased capacity of antioxidant defence, therefore, having anti-inflammatory effects in patients with T2DM.[72,73] It was demonstrated that obesity and increased BFP may lead to an increase in the concentration of TNF-α; therefore, exercise induces a reduction in the concentration of TNF-α by enhancing fat loss.[13] In addition, it seems that exercise training may be an effective strategy in lowering systematic inflammation by reducing BFP.[14] In the present study, BM and BFP are significantly decreased in the three intervention groups (CTS, CT and S), and the most significant reduction was observed in the CTS group. Also, there was a correlation between inflammatory markers and BFP, suggesting that reductions in TNF-α, IL-6, hs-CRP and IL-1β could be in part attributed to changes in body fat.

Data from the present study indicated that the CT group experienced a significant decrease in LBM following 12 weeks of CT. The occurrence of interference effect might be inevitable when RT and AT are combined in a single training session, especially without resting intervals (at least 6 h) between AT and RT modalities, which can increase muscle protein breakdown (MPB) while decreasing muscle protein synthesis (MPS).[65] Based on the molecular perspective, since the higher intensity of AT protocol is associated with increased glycogen depletion, which may exacerbate residual fatigue, it is highly likely that it reduced the activity of the mechanistic target of rapamycin (mTOR) activity by increasing the AMPK signalling[65] in the present study. These effects can reduce the gains in LBM. Although we used a high intensity interval training (HIIT)-based protocol to reduce the interference effect as recommended recently,[74] this did not preclude LBM decrements. Furthermore, protein intake is highly beneficial for muscular gains (strength, hypertrophy, power, etc.). For boosting RT gains, protein intakes of ~1.6–2.2 g/kg/day have been recommended.[75–77] In the only study on CT, 2 g/kg/day of protein following 12 weeks of CT successfully minimized the interference effect and increased total LBM gains by 4% in recreationally active males.[78] In this study, our participants consumed less than 1 g/kg/day, which was not sufficient to at least maintain LBM values. Therefore, a decrease in LBM is not a surprising outcome in the present study.

Based on the results of the present study, there was a beneficial additive effect of saffron supplementation in conjunction with concurrent training on improving inflammatory markers. The potential mechanisms for these changes may be attributed to the synergetic interaction of saffron metabolites, especially carotenoids (crocin and crocetin) and polyphenols with the increase of the enzymatic antioxidant defence system induced by concurrent training. However, the precise underlying mechanism(s) necessitates further research.

Our results should be interpreted taking into consideration the following limitations. First, although in our study the metabolite values of saffron used are known, we assessed the whole saffron as an anti-inflammatory agent; therefore, we could not elucidate to what extent each of the available biological components of saffron possesses anti-inflammatory effects. Second, self-reported data were used to monitor dietary intake and, therefore, dietary intake was not precisely controlled. Third, bioelectrical impedance was utilized to measure body composition, an approach not as precise as dual-energy X-ray absorptiometry or hydrostatic weighing (gold standards in the assessment of body composition); however, prior research has shown BIA, when performed in a controlled environment, to be a valid and reliable method.[79] Also, the present study only assessed nutrient intake before and after the intervention period. Another nutritional assessment in the middle of the intervention would have provided clearer dietary monitoring.

5 | CONCLUSION

In conclusion, the additive effect of saffron supplementation and concurrent training led to a greater improvement in inflammatory markers, body composition and glycaemic markers compared to the saffron supplementation or concurrent training alone. This treatment can be used as an effective method to improve T2DM-related metabolic abnormalities.

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COMPETING INTERESTS

The authors declare no conflicts of interest.

CONTRIBUTORS

B.H.M., A.A.G. and M.K. conducted the formal analysis. B.H.M., A.A.G., M.K. and S.R.A. carried out the investigations. All authors were involved in the writing of the original draft. A.R. was responsible for the project administration.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Some data may not be made available because of privacy or ethical restrictions.

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