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High-intensity interval training with or without chlorella vulgaris supplementation in obese and overweight women: effects on mitochondrial biogenesis, performance and body composition

Mahzad Sanayei[1,2], Fatemeh Hajizadeh-Sharafabad[1,2], Ramin Amirsasan[3] and Ali Barzegar[4]*

[1]*Department of Community Nutrition, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*
[2]*Student Research Committee, School of Nutrition & Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*
[3]*Associate Professor in Exercise Physiology and Sport Nutrition, University of Tabriz, Tabriz, Iran*
[4]*Nutrition Research Center, Department of Community Nutrition, School of Nutrition & Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran*

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Abstract

The beneficial effects of high-intensity interval training (HIIT) and chlorella vulgaris (CV) on body composition and mitochondrial biogenesis have been shown in some mechanistic studies. This study aimed to determine the effects of CV and/or HIIT on mitochondrial biogenesis, performance and body composition among overweight/obese women. There was a significant reduction in the fat mass (FM) of the CV + HIIT group, as compared with the placebo group ($P = 0{\cdot}005$). A marginal significant increase in body water ($P = 0{\cdot}050$) and PPAR-γ coactivator-1α ($P = 0{\cdot}050$) was also found only in the CV + HIIT group, as compared with the placebo. Relative ($P < 0{\cdot}001$) and absolute ($P < 0{\cdot}001$) VO_{2max}, as well as Bruce MET ($P < 0{\cdot}001$), were significantly increased in the HIIT and HIIT + CV groups. Besides, the synergistic effect of CV and HIIT on the Bruce MET increment was found (interaction P-value = 0·029). No significant changes were observed in BMI, fat-free mass, visceral fat, silent information regulator 1 and fibroblast growth factor-21. In this randomised clinical trial, forty-six overweight/obese women were assigned to four groups including CV + HIIT and HIIT + placebo groups that received three capsules of CV (300 mg capsules, three times a day) or corn starch, in combination with three sessions/week of HIIT. CV and placebo groups only received 900 mg of CV or corn starch, daily, for 8 weeks. Biochemical assessments, performance assessment and body composition were obtained at the beginning and end of the intervention. HIIT may be, therefore, effective in improving mitochondrial biogenesis, performance and body composition in overweight/obese women.

Key words: **High-intensity interval training (HIIT): Mitochondrial biogenesis: Body composition: Performance**

Total body fat is associated with impaired mitochondrial function, thus indicating a strong relationship between body composition and mitochondrial energy metabolism[1]. Mitochondria, as an important cell organelle, is involved in many crucial cell functions such as metabolism, regulating the maximal oxygen consumption (VO_{2max}), which is important for endurance performance[2]. VO_{2max} is the maximum (max) amount of oxygen (O_2) a person utilises during his/her exercise; it is considered as a common measurement of aerobic power. Some characteristics including sex, age, body composition, exercise history and diet can affect VO_{2max}[3].

Endurance exercise-induced adaptations in mitochondrial activity can improve the metabolic health and decrease the risk of obesity and other metabolic disturbances[4]. Higher mitochondrial biogenesis is associated with aerobic performance as well as muscle oxidative capacity and regulated by transcriptional cofactors such as PPAR-γ coactivator-1α (PGC-1α)[5]. Deacetylation of PGC-1α by silent information regulator 1 (SIRT1) which is an NAD-dependent deacetylase increases PGC-1α activity, resulting in the activation of mitochondrial biogenesis[6,7]. Besides, the gene expression of PGC-1α is up-regulated via fibroblast growth factor-21 (FGF-21)[8].

Abbreviations: CV, chlorella vulgaris; EPOC, exercise oxygen consumption; FFM, fat-free mass; FGF-21, fibroblast growth factor-21; FM, fat mass; HR, heart rate; HIIT, high-intensity interval training; LBM, lean body mass; max, maximum; PGC-1α, PPAR-γ coactivator-1α; SIRTI, silent information regulator 1.

* **Corresponding author:** Ali Barzegar, email barzegarali@tbzmed.ac.ir

During exercise, SIRT1 is increased, subsequently activating PGC-1α[9]. High-intensity interval training (HIIT), a time-efficient strategy done above 85 % of the maximal aerobic capacity, can increase the skeletal muscle adaptation and exercise capacity via some cellular pathways including mitochondrial biogenesis[10]. Based on the American College of Sports Medicine's Worldwide Survey of Fitness Trend for 2019, HIIT was in the five top trends for fitness from 2014 to 2020[11].

In a comprehensive review, the HIIT's effect on the body composition was investigated, showing a significant reduction in the whole-body FM and waist circumference in the obese individuals, even in the absence of body weight change[12]. Body fat accumulation could decrease the relative VO_{2max}; thus; more FM could be, therefore, a better predictor of relative VO_{2max}, as compared with exercise performance tests[13].

Dietary antioxidants with electron-donor properties such as vitamin C, vitamin E, riboflavin, Q10 and α-lipoic acid can also stimulate mitochondrial biogenesis, as well as VO_{2max}[14]. Chlorella vulgaris (CV), as a well-known algae, has been recently considered as a valuable functional food due to the high content of vitamins, minerals and phenolic components[15]. CV has been shown to have antioxidant, anti-inflammatory and immunomodulatory properties[15]; its high antioxidant content makes it an appropriate supplement for the exercising people.

According to our research, no previous study had yet investigated the combined effect of HIIT and CV supplementation on mitochondrial biogenesis and performance in the overweight or obese women. Therefore, this study aimed to evaluate the effects of HIIT and/or CV, as well as any synergic effect of these two interventions, on PGC-1α, FGF-21, SIRT1, body composition and performance parameters.

Materials and methods

Participants

This study was performed in Tabriz, Iran, from June 2019 to November 2019. Participants were recruited from Tabriz area via flyers throughout the community and healthcare centres. Obese or overweight women, aged 18–35 years, with a BMI ranging from 25 to 35 kg/m^2 were included in the study. Main exclusion criteria were weight fluctuations more than 3 kg during the last 6 months, continuous physical activity, joint disabilities, severe health conditions, consumption of antioxidant supplements and a particular diet. The study protocol was approved by the ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1397·922). In an awareness condition, participants signed the informed consent. This clinical trial was registered at the Iranian Registry of Clinical Trials, (https://www.irct.ir/trial/37816, with the Registration number: IRCT20190224042821N1).

Study design

This study was an 8-week randomised, double-blind, placebo-controlled trial involving four treatment groups: CV, HIIT+placebo, CV + HIIT and placebo. CV group received CV powder with 98 % purity (300 mg capsules, three times a day); placebo group got the corn starch powder (300 mg capsules, three times a day); HIIT + CV group received a similar amount of the CV powder in combination with three sessions/week of HIIT; and also HIIT+placebo group received similar amounts of the placebo in combination with three sessions/week of HIIT in daily manner, for 8 weeks. Randomisation for individual assignments in each group was done using computer-generated random numbers by a statistician. The intervention allocation was blinded for participants and investigators. The blinding was maintained throughout the study as well as during statistical analysis. The codes for the jars were kept under locked storage by a researcher with no involvement in the trial or statistical analyses. Pure CV powder (with the purity of 98 % net weight) was supplied from a knowledge-based company (Riz Jolbaki Parsian). Each CV capsule was filled with 300-mg CV powder and placebo of corn starch; this was done in a pharmacological laboratory, under a sterile condition (Baharan pharmacology company). The dosage of CV was determined based on the previous studies that had shown the proper tolerability, safety and the effectiveness of CV in improving the metabolic parameters[16–18].

The dietitian visited the participants at the baseline and biweekly; this was done till the end of intervention to evaluate the compliance of the participants to the supplements; they were asked about possible adverse effects. In these intervals, volunteers were asked to bring their jars back and the remaining capsules were counted. The participants with a compliance below 90 % were omitted. In addition, the dietary intake was evaluated using the dietary food record (three non-consecutive days) before and after the intervention by Nutritionist 4 software; the subjects were requested not to change their dietary pattern. Participants were instructed to record the amount of the consumed foods and beverages in the portion size and volumes, so that they could be defined correctly.

Anthropometric and body composition measures

The anthropometric parameters were assessed at the beginning and after the 8-week intervention. Height measurement was done via a wall-mounted Stadiometer (Seca) with 0·1-cm accuracy. Body weight was measured after some overnight fasting with the least clothing, using an identical scale (Seca) with 0·1-kg accuracy. Body composition comprising fat mass (FM) and fat-free mass (FFM) was assessed using a bioimpedance analyser (Tanita MC-780 S MA).

Blood sampling and analysis

Blood samples from the medial cubital vein were taken after some overnight 8- to 12-h fasting and centrifuged at 4°C for 15 min to isolate the plasma from whole blood. Plasma samples were aliquoted and stored at −80°C until the biochemical analyses. Plasma levels of PGC-1α, SIRT-1 and FGF-21 were assessed using the ELISA method via commercial kits (Shanghai Crystal Day Biotech Company). According to an excellent correlation between muscle and serum PGC-1α levels, the minimally invasive method of assessment (blood sampling instead of biopsy) was chosen[19,20].

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Bruce performance test

The Bruce protocol is a maximal exercise test developed by Robert A. Bruce; it can evaluate the cardiac health status[21]. Before the study, subject's familiarisation with the HIIT protocol was ensured. The protocol consisted of multiple three min exercise stages in which the gradient and speed of each stage were increased in the treadmill. For example, stage 1 was 1·7 mph at a 10 % gradient; the second one was 2·5 mph at a 12 % gradient and the third one was 3·4 mph at a 14 % gradient; they were increased in a step-by-step manner. Before the test started, subjects warmed up with the help of an expert trainer. During the test, the trainer reminded the subjects to have normal breath and encouraged them till the point of exhaustion, such that they could not continue and treadmill was stopped. The instrument used in this study was Technogym treadmill (Cesena) without oxygen mask, with a computer program for the test performance. Relative VO_{2max}, absolute VO_{2max} and Metabolicequivalent of task (MET), max heart rate (HR), total energy burn and time were the outputs calculated via the computer program-defined formula.

High-intensity interval training protocol

In this study, HIIT was a running-based prescription aimed to achieve a target max HR per week. Throughout the study, an expert trainer supervised the HIIT programme. Three exercise sessions per week were conducted over the 8 weeks of trial, giving a total of twenty-four scheduled sessions. Individualisation of the training programme was performed using the max HR of the participants; the progression of the exercise intensity was provided by an increase in the HR in the course of the study (Table 4).

Exercise intensity was determined based on Bruce test outcomes, which included the max HR and VO_{2max} of the participants. Weekly target HR zone for each participant was calculated using the Karvonen formula, as follows:

$$\text{Target heart rate} = [(\text{max HR} - \text{resting HR}) \times \%\,\text{Intensity}] + \text{resting HR}.$$

To control the individualised max HR during exercise, telemetry (Pollar) was applied. Each session began and ended with 10-min warming up and 10-min cooling down. The intensity of the HIIT sessions was designed to increase gradually over 8 weeks. Accordingly, HIIT, done three times per week, was commenced with 20 × 40 s protocol (intensity of 50–60 % max HR) at the first week; this was followed by 20 × 35 s (intensity of 60–70 % max HR) in the second week, 16 × 30 s (intensity of 70–80 % max HR) in the third week, 16 × 25 s (intensity of 80–90 % max HR) in the fourth week and 12 × 20 s (intensity of 90–100 % max HR) in the last month (online Supplementary Table 1).

Statistical analysis

Considering $\alpha = 0{\cdot}05$, 95 % confidence and power of 80 %, the sample size was calculated to be nine subjects in each group, based on the fasting blood sugar (as secondary outcome) extracted from the previous investigations[16]. By anticipating a 20 % dropout rate, totally, we included forty-six subjects in the study: twelve and eleven participants in every two arms. Data analyses were performed using the intention-to-treat method on all subjects who were included (n 46) in the study. To run intention-to-treat analyses, missing data were imputed based on the Last-Observation-Carried-Forward (LOCF) method. To examine the normal distribution of the variables, the Kolmogorov–Smirnov test was applied. For the analysis of the categorical variables comparison, χ^2 test was used. As all quantitative variables had normal distribution, paired samples t test and one-way ANOVA with Bonferroni *post hoc* pairwise analyses were used for within-group and between-group comparisons, respectively. ANCOVA adjusted for baseline values were applied to evaluate the absolute effect of treatments on body composition constituents, biogenesis biomarkers and performance parameters.

Moreover, to evaluate the effects of CV and HIIT independently (the main effects of CV or HIIT), as well as the possible synergistic effects of these treatments (interactions between CV and HIIT), a 2 × 2 factorial design was applied:

- CV (CV and CV + HIIT groups) *v.* no CV (HIIT+ placebo and placebo groups)
- HIIT (HIIT and CV + HIIT groups) *v.* no HIIT (CV and placebo groups). SPSS software, version 23, was used for statistical analyses and significance level was assumed at P-values less than 0·05.

Results

Participants' characteristics

A flow diagram of the study subjects is presented in Fig. 1. Of the forty-six participants initially enrolled in the study, two in the CV group did not complete the study because of poor compliance and abdominal cramps. Also, two subjects in the placebo group did not complete the study due to personal reasons either. Therefore, forty-two participants finished the 8-week intervention period. Eventually, based on the intention-to-treat principle, all of forty-six participants were included in the analyses. No serious adverse effects following CV supplementation were reported by the subjects. Only one person reported abdominal cramps.

Body composition

Baseline values of body weight, FM, FFM, lean body mass (LBM), body water and visceral fat were not different among the groups. Based on the ANOVA or ANCOVA tests, CV intake or HIIT had no significant effect on FM, while the combination of CV and HIIT significantly decreased FM, as compared with the placebo group (–5 % in CV + HIIT *v.* 1 % in the placebo, $P = 0{\cdot}005$). The 2 × 2 factorial analysis adjusted for the baseline values also demonstrated that CV supplementation decreased FM, as compared with no CV (–3 % in CV *v.* 0·4 % in no CV, $P = 0{\cdot}003$). Similarly, the HIIT group showed a significant reduction in FM, as compared with the one getting no HIIT (–3 % in the HIIT group *v.* 0 % in the one getting no HIIT, $P = 0{\cdot}015$). However, the interaction effect was not statistically significant.

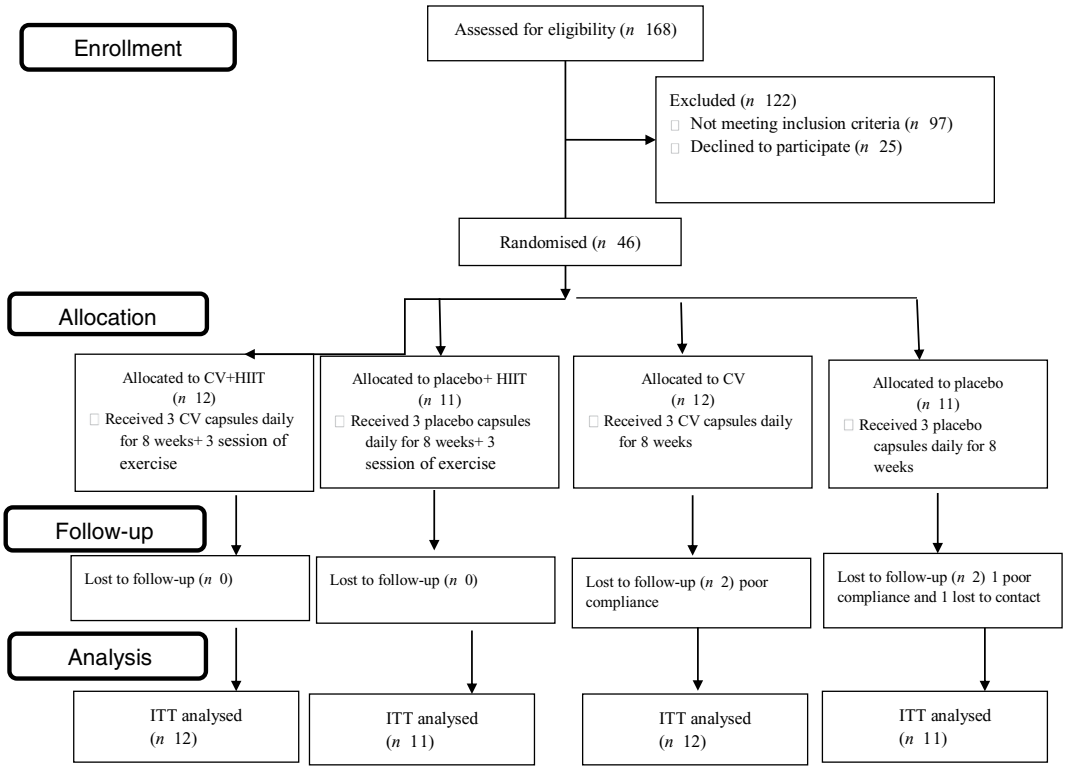

Fig. 1. Flow diagram of study participants.

The combination of CV and HIIT led to a marginal significant increase in the body water percentage, as compared with the placebo group (28 % in CV + HIIT *v.* −16·4 % in the placebo, $P = 0{\cdot}050$). The 2 × 2 factorial analysis adjusted for the baseline values also showed a significant increase in the body water of the HIIT group, as compared with the one getting no HIIT (1·33 % in HIIT *v.* −1·01 % in the one getting no HIIT, $P = 0{\cdot}043$), while the interaction effect between CV and HIIT was not significant. Neither ANOVA/ANCOVA nor factorial analysis, however, showed any significant between-group differences in body weight, FFM, LBM and visceral fat (Table 1).

Mitochondrial biogenesis factors

Baseline values of PGC-1*α*, SIRT1 and FGF-21 were not different in the groups. ANOVA and ANCOVA showed a marginal significant increase in the PGC-1*α* level in CV (64 %), HIIT (54 %) and CV + HIIT (73 %) groups, as compared with the placebo (3 %). The 2 × 2 factorial analysis adjusted for the baseline values also indicated that HIIT significantly increased the PGC-1*α* levels in comparison with the no HIIT one (63 % in HIIT *v.* 33 % in no HIIT, $P = 0{\cdot}047$), while CV supplementation did not lead to any significant changes in PGC-1*α* levels, as compared with no CV. In addition, the synergistic effects of CV and HIIT on PGC-1*α* levels were not significant due to the lack of a significant interaction effect ($P = 0{\cdot}290$). In fact, neither ANOVA nor 2 × 2 factorial analysis indicated any significant between-group differences in SIRT1 and FGF-21 changes (Table 2).

Performance parameters

At the baseline, no significant differences were observed in HR, Bruce MET, relative VO_{2max} and absolute VO_{2max}. However, ANCOVA adjusted for the baseline values showed significant between-group differences in Bruce MET, relative VO_{2max} and absolute VO_{2max} after 8 weeks. Based on the results obtained from Bonferroni *post hoc* pairwise comparisons, only CV + HIIT significantly increased the Bruce MET (19 % in CV + HIIT *v.* 3 % in the placebo, $P < 0{\cdot}001$), as compared with the placebo group. The 2 × 2 factorial analysis adjusted for the baseline values also revealed the synergistic effect of HIIT and CV on the Bruce MET due to a significant interaction effect ($P = 0{\cdot}029$).

Regarding the relative VO_{2max}, CV + HIIT (18 % in CV + HIIT *v.* −0·3 % in the placebo) and HIIT (14 % in CV + HIIT *v.* −0·3 % in the placebo) groups showed a significant increase, as compared with the placebo. Furthermore, CV + HIIT (17 % in CV + HIIT *v.* 1 % in the placebo) and HIIT (14 % in CV + HIIT *v.* 1 % in the placebo) groups displayed a significant increase in absolute VO_{2max}, as compared with the placebo. Based on 2 × 2 factorial analyses, HIIT showed a significant increase in relative VO_{2max} (18 % in HIIT *v.* 3 % in no HIIT, $P < 0{\cdot}001$) and absolute VO_{2max} (18 % in HIIT *v.* 0·6 % in no HIIT, $P < 0{\cdot}001$), as compared with no HIIT. The synergistic effects of HIIT and CV on relative VO_{2max} ($P = 0{\cdot}846$) and absolute VO_{2max} ($P = 0{\cdot}323$) were, however, not significant due to the lack of significant interaction effects. Neither ANOVA nor 2 × 2 factorial analysis showed any significant between-group differences in HR changes (Table 3).

Table 1. Body composition characteristics of study participants at baseline and after 8 weeks of intervention (Mean values and standard deviations; mean difference and 95 % confidence interval)

	HIIT + CV (*n* 12)			HIIT + Plc (*n* 11)			CV (*n* 12)			Plc (*n* 11)				
Variable	Mean	SD	*P*§	Mean	SD	*P*§	Mean	SD	*P*§	Mean	SD	*P*§	*P*‡	*P*† Interaction (CV × HIIT)
Height (cm)	162·5	8·22		159·63	5·4		163·54	4·6		160	4·75		0·332	–
Weight (kg)														
Before	82·15	13·53		76·3	6·83		79·13	9·84		73·3	6·95		0·734	0·678
After	82·06	14·87		75·7	6·76		79·15	9·52		73·44	10·43			
MD	–0·21		0·889	–0·54		0·361	–0·02		0·968	0·7420·28		0·742		
95 % CI	–1·26, 0·83			–1·61, 0·52			–1·04, 1·0			–0·81, 1·37				
FM percentage														
Before	37·67	3·62		35·84	2·84		35·91	2·43		34·30	1·85		0·005*	0·318
After	35·72	5·29		35·63	2·56		35·39	1·80		34·72	2·13			
MD	–2·04		0·012*	–0·20		0·553	–0·51		0·211	0·52		0·098		
95 % CI	–2·96, –1·12			–1·12, 0·71			–1·39, 0·36			–0·44, 1·48				
FFM percentage														
Before	50·30	5·26		49·29	3·17		50·92	4·59		49·40	3·75		0·077	0·784
After	51·30	6·13		49·55	3·20		51·10	4·00		48·74	4·13			
MD	1·22		0·071	0·24		0·682	0·20		0·716	–0·67		0·138		
95 % CI	0·24, 2·19			–0·77, 1·26			–0·78, 1·18			–1·69, 0·35				
LBM percentage														
Before	54·41	1·71		53·11	1·04		55·58	1·77		52·49	1·39			0·749
After	55·40	1·97		53·34	1·04		55·83	1·49		51·70	1·50			
MD	0·99		0·076	0·23		0·715	0·18		0·717	–0·64		0·158	0·174	
95 % CI	–0·12, 2·11			–1·15,1·62			–0·87,1·23			–1·58,0·29				
Body water (%)														
Before	44·50	3·01		46·83	2·47		46·53	3·31		48·80	2·55			
After	45·80	4·22		46·69	2·33		46·32	3·15		47·98	2·52			
MD	1·29		0·032*	–0·14		0·676	–0·21		0·712	–0·81		0·045*	0·05	0·374
95 % CI	0·13, 2·45			–0·89, 0·60			–1·48, 1·06			–1·59, –0·02				
Visceral fat (%)														
Before	6·08	1·92		5·36	1·85		5·22	1·39		4·66	1·65			0·339
After	5·66	2·10		5·54	1·86		5·33	1·22		5·00	1·73		0·064	
MD	–0·41		0·096	0·18		0·167	0·11		0·594	0·33		0·08		
95 % CI	–0·92, 0·08			–0·08, 0·45			–0·35, 0·57			–0·05, 0·71				

CV, chlorella vulgaris; Plc, placebo; FFM, fat-free mass; FM, fat mass; LBM, lean body mass; HIIT, high-intensity interval training.
* *P*-value significance level < 0·05.
† *P* based on for two-way ANOVA adjusted for baseline values.
‡ *P* based on ANCOVA test adjusted for baseline values.
§ *P* based on paired samples *t* test for intragroup comparisons.

Table 2. Mitochondrial biogenesis factors of study participants at baseline and after 8 weeks of intervention (Mean values and standard deviations; mean difference and 95 % confidence interval)

	HIIT + CV (*n* 12)			HIIT + Plc (*n* 11)			CV (*n* 12)			Plc (*n* 11)				
Variable	Mean	SD	*P*§	Mean	SD	*P*§	Mean	SD	*P*§	Mean	SD	*P*§	*P*‡	*P*† Interaction (CV × HIIT)
PGC-1α (ng/ml)														
Before	11·56	10·38		12·51	11·24		9·82	10·18		10·91	11·56		0·05	0·29
After	19·99	12·80		19·33	10·05		16·26	14·94		11·25	11·46			
MD	8·45		0·001*	6·88		0·001*	6·39		0·076	0·34		0·129		
95 % CI	4·31,12·58			2·55,11·21			2·24,10·53			2·24,10·53				
SIRT-1 (pg/ml)														
Before	24·98	21·06		26·97	31·72		22·94	27·75		32·10	47·00		0·151	0·86
After	35·18	30·82		30·62	34·09		27·65	31·80		31·57	43·68			
MD	10·23		0·069	3·65		0·183	4·78		0·063	–0·64		0·744		
95 % CI	3·76,16·70			–3·11,10·41			–1·70,11·26			7·42,6·14				
FGF-21 (ng/ml)														
Before	973·14	910·66		950·31	1001·45		926·26	1124·04		709·81	836·99		0·093	0·365
After	649·29	614·66		692·32	680·94		728·94	729·77		836·30	943·66			
MD	–297·87		0·016*	–239·37		0·130	–186·43		0·283	67·64		0·188		
95 % CI	–504·71, –91·03			–455·30, –23·44			–393·11, 20·24			–149·17, 284·46				

CV, chlorella vulgaris; Plc, placebo FGF-21, fibroblast growth factor 21; HIIT, high-intensity interval training; PGC-1α, PPAR-γ coactivator-1α; SIRT-1, sirtuin 1.
* *P*-value significance level < 0·05.
† *P* based on for two-way ANOVA adjusted for baseline values.
‡ *P* based on ANCOVA test adjusted for baseline values.
§ *P* based on paired samples *t* test for intragroup comparisons.

Discussion

To the best of our knowledge, the present clinical trial is the first one assessing the effect of HIIT (running protocol), alone or in combination with CV, on the body composition, performance test outputs and some mitochondrial biomarkers in the overweight and obese subjects. First, we hypothesised that HIIT and CV supplementation would improve body composition. In this regard, a significant reduction in FM was observed following CV supplementation plus HIIT, as compared with other groups. In addition, body water was significantly increased in the HIIT group, in comparison with the placebo. However, neither CV nor HIIT, alone or in combination with each other, led to any significant changes in FFM. In an RCT, as done by Mizoguchi *et al.*, supplemented with CV (40 tablets/d) for 16 weeks, the reducing effect of CV on body fat reduction was attributed to a gene named protein tyrosine phosphatase 1B (PTP-1B), a negative regulator of insulin pathway and leptin[22]. Besides this, the chlorophyll content of CV may decrease the FM via suppressing adipogenesis and activating white adipose tissue browning[23,24]. The chlorophyll-containing parts of CV possess various antioxidants like carotenoids; they have a regulatory role in the adipose tissue biology, such as adipogenesis, adipocytes metabolism and secretory actions, as confirmed in several studies[25,26]. Polyphenolic contents of CV, such as catechins, epigallocatechin gallate, flavonols and flavones, may also inhibit preadipocyte differentiation and decrease the fat accumulation[27,28]. Meanwhile, the magnitude of reduction in the FM following the supplementation of CV alone was smaller than that required to reach a significance level, which could be due to supplementing the low dose of CV for the relatively short intervention period. Regarding the effect of HIIT on body composition, a systematic review and meta-analysis indicated that the average 10 weeks of HIIT could reduce FM about 2·6 kg (~ 10 % decrease), even in the absence of body mass changes[12]. Interestingly, the recent investigation also showed significant improvements in body composition and aerobic capacity in the overweight men when following HIIT once a week, which was much less than the usual levels (the usual protocol for HIIT consists of running three times a week)[29].

Energetic restriction is the most effective way for weight loss, while exercise is more influential on the visceral fat reduction[30]. In addition, a greater weight loss was observed during energy restriction, which was partly related to a marked reduction in LBM and muscle wasting. Considering that LBM is the most metabolic active part determining energy expenditure, LBM reduction resulted in a significant reduction of the BMR, making it hard to reach the appropriate weight loss[31]. Thus, all weight loss plans should be judged in terms of their success in achieving the body fat loss instead of body weight or BMI. In this regard, HIIT-induced weight loss, which was accompanied by fat loss and preservation of lean mass, led to great health benefits[32]. The mechanisms underlying FM reduction following HIIT are not fully known; despite this, one of the most probable ones is an increase in the excess post-exercise oxygen consumption (EPOC), also known as HIIT-induced EPOC, which is unique to this form of exercise[33]. In a study investigating the EPOC differences between continuous and interval training, the higher EPOC in interval training, in comparison with the continuous one, was detected, thus suggesting that the former led to more body fat loss for a given amount of energy expenditure. This also showed that the magnitude of EPOC and its duration depended on exercise intensity, which was much higher in the interval training[34].

The second hypothesis regarding the effect of HIIT and CV on performance capacity was confirmed only for HIIT and HIIT plus CV supplementation, which significantly increased the Bruce MET, and relative and absolute VO_{2max} levels. The synergistic effect of HIIT and CV on the Bruce MET was also observed. In the obese or overweight subjects, especially in those with central obesity, mechanical power was decreased, thus preventing the individuals from reaching the maximal ability in the VO_{2max} test. Unbalanced body composition could also decrease VO_{2max} in the obese subjects[35].

Training intensity can determine the alteration in the electron transport system. On the other hand, the maximal electron transfer via flavoproteins can increase the fat oxidation[36]. In the present study, HIIT increased the absolute VO_{2max} up to 14 %. Although VO_{2max} was increased in response to the HIIT (more common in training up to 60 min with more than 65 % intensity), according to physiological improvements, the skeletal muscle respiratory capacity was increased primarily in the fat oxidation capacity area[37].

Consistent with the present study, the results obtained from a systematic review indicated that 2 to 15 weeks HIIT in healthy young and older adults significantly increased VO_{2max} (from 4 % to 46 %). While the mechanisms of HIIT that lead to modulating this aerobic fitness improvement are unclear, the most likely one is phosphocreatine degradation during these intervals. The major energy source for this kind of exercise is ATP; repeated intervals in HIIT can lead to the progressive increase in oxidative ATP generation[38]. Due to the short period of high-intensity exercise with rest intervals in HIIT, local metabolic capacities including creatine phosphate, ATP and mitochondrial enzymes can supply the growing energy demands, and the recovery times may compensate the depleted energy. Another probable mechanism of HIIT is related to the increased stroke volume that is induced by cardiac contractility enhancement, mitochondrial oxidative capacity improvement and skeletal muscle diffusive ability[39]. This aerobic capacity encasement is also related to AMP-mediated PGC-1α up-regulation, which is responsible for oxidative adaptation and the remarkable increase in the maximal oxygen uptake after HIIT[40].

The synergistic effect of CV supplementation on HIIT is probably due to the decreased FM. In other words, the significant improvement in body composition that resulted from CV supplementation led to an increase in energy expenditure during the exercise. Considering that obese or overweight individuals often experience cell hunger, which is a kind of malnourishment, CV with a variety of nutrients can also compensate this deficiency and increase the aerobic capacity[41].

The third hypothesis was that HIIT and CV might improve the serum levels of PGC-1α, SIRT-1 and FGF-21 as the mitochondrial biogenesis indicators. Our interventions did not result in any statistically significant improvement in SIRT-1 and FGF-21 *v.* placebo; despite this, we did demonstrate the efficacy of using

Table 3. Performance parameters of study participants at baseline and after 8 weeks of intervention
(Mean values and standard deviations; mean difference and 95 % confidence interval)

	HIIT + CV (*n* 12)			HIIT + Plc (*n* 11)			CV (*n* 12)			Plc (*n* 11)				
Variable	Mean	SD	*P*§	Mean	SD	*P*§	Mean	SD	*P*§	Mean	SD	*P*§	*P*‡	*P*† Interaction (CV × HIIT)
Bruce MET														
Before	9·63	2·59		10·81	1·35		9·21	2·36		11·04	1·07		< 0·001*	0·029*
After	11·50	2·36		11·74	1·97		9·31	2·37		11·36	1·51			
MD	1·85		<0·0001*	0·96		0·005*	0·06		0·413	0·35		0·277		
95 % CI	1·33,2·36			0·42,1·50			–0·46,0·60			–0·19,0·90				
Relative VO_{2max} (ml o_2/kg)														
Before	33·74	9·01		37·40	4·77		32·96	4·79		38·81	3·85		< 0·001*	0·846
After	40·51	8·26		42·59	4·51		35·48	4·65		37·96	3·13			
MD	6·35		< 0·0001*	5·58		0·001*	1·93		0·218	–0·14		0·373		
95 % CI	4·58,8·12			3·74,7·42			0·14,3·73			–2·03,1·75				
Absolute VO_{2max} (L o_2/min)														
Before	2·78	0·61		2·84	0·34		2·52	0·68		2·92	0·41		< 0·001*	0·323
After	3·27	0·47		3·21	0·23		2·54	0·64		2·89	0·38			
MD	0·49		0·015*	0·40		< 0·0001*	–0·07		0·609	0·03		0·810		
95 % CI	0·31,0·68			0·21,0·59			–0·26,0·12			–0·17,0·22				
Maximum HR														
Before	175·91	4·71		172·18	6·02		174·50	3·62		179·54	3·63		0·783	0·128
After	185·41	2·84		174·81	6·26		178·58	3·42		180·54	2·26			
MD	–9·50		0·076	–2·64		0·812	–4·08		0·220	–1·00		0·775		
95 % CI	–20·16,1·16			–26·73,21·46			–10·99,2·83			–8·58, 6·58				

CV, chlorella vulgaris; Plc, placebo; HIIT, high-intensity interval training; HR, heart rate.
* *P*-value significance level < 0·05.
† *P* based on for two-way ANOVA adjusted for baseline values.
‡ *P* based on ANCOVA test adjusted for baseline values.
§ *P* based on paired samples *t* test for intragroup comparisons.

Table 4. Training programme in 8 weeks

Weeks	Intesity according to HRR	Time of activity (min)	Time of active rests (min)	Frequency (sets, repetition)	Time of protocol (min)	The total duration of activity (main activity, warm-up and cool down) (min)
First	50–60 %	13·33	11·50	5, 4	24·83	44·83
Second	60–70 %	11·66	13·41	5, 4	25·07	45·07
Third	70–80 %	8	13·33	4, 4	21·33	41·33
Fourth	80–90 %	6·66	15	4, 4	21·66	41·66
Five to eight	90–100 %	4	14·16	3, 4	18·16	38·16

CV in enhancing the PGC-1α level in combination with exercise. Antioxidant stimulus may be required to go along with training to stimulate PGC-1α, as the previous studies had demonstrated that an antioxidant compound could be useful to up-regulate the gene expression of PGC-1α during exercise[42,43,20]. Overall, for HIIT or CV supplementation to have enough efficacy on mitochondrial biogenesis, it should be intervened for a time interval longer than that used in the current study. In addition, the lack of a significant effect on mitochondrial biogenesis parameters following CV or HIIT was presumably due to the within-normal range of baseline values among the participants.

No study had yet investigated the combined effect of CV and HIIT on the mitochondrial biogenesis; despite this, there are some studies on the individual effect of CV or HIIT. PGC-1α has been considered as the main contributor to oxidative adaptation following exercise; the recent studies have also shown that the acute HIIT can increase PGC-1α mRNA[44]. In a study on obese individuals, the 12-week HIIT intervention induced an approximately threefold increase in PGC-1α[45]. In another study, subjects performed normal intensity training for 4 weeks; this was then followed by 40 sessions of HIIT, twice, in a daily manner, for 20 consecutive days; however, there was no significant change in the nuclear or cytosolic content of PGC-1α. The authors cited the much larger number of training sessions and a more reduction in the relative exercise intensity between the pre- and post-HIIT trials as a possible reason for the insignificant changes in PGC-1α[46].

In the present study, SIRT-1 change, after 8 weeks of HIIT and/or CV, was insignificant; this was similar to the results of another study on recreationally active men under a 6-week low-volume HIIT[47]. However, in the study done by Little *et al.*, the 2 weeks HIIT significantly enhanced the SIRT-1content. Similar to our results, Jasmin *et al.* showed a significant increase in PGC-1α, without any marked change in SIRT-1 level following the 4-week HIIT[48]. In another study, the 6-week HIIT increased the mitochondrial enzymes activity, PGC-1α and SIRT-1[49].

While obesity can increase FGF-21, a browning mediator in the adipose tissue, exercise may reduce its level; nevertheless, neither HIIT nor CV caused a significant change in plasma FGF-21 in the current study. In the study of some overweight and obese young men, a 6-week HIIT did not lead to any significant changes in FGF-21. In contrast, the 8-week HIIT in the obese men decreased FGF-21 along with insulin resistance[50]. Surprisingly, in another study on sedentary obese women, the same intervention resulted in a significant increase in FGF-21[51]. These discrepancies may be related to the much greater difference in exercise protocols and its duration, the baseline physical fitness of the individuals, health status, and sex. In the muscle cells, PGC-1α expression is induced in response to Ca signalling by Ca^{2+}/calmodulin-dependent protein kinase 4 (CaKMIV) and calcineurin A (CnA). Phosphorylation of CaMKIV and the subsequent activation of the cAMP response element (CRE)-binding protein (CREB) can stimulate PGC-1α expression during exercise[52]. On the other hand, phosphorylation of activated protein kinase (AMPK) can not only directly activate PGC-1α but may also cause SIRT1-mediated PGC-1α activation[53]. The activation of this pathway results in the adipocyte size reduction, lipid oxidation in mitochondria and mitochondrial density increment, which can all promote the max oxygen consumption[54]. The other exercise-induced mechanism for PGC-1α expression is the activation of p38 mitogen-activated protein kinase (p38 MAPK)[55].

Strengths and limitations

The regular attendance of subjects in exercise sessions and individualised exercise protocol according to the max HR and in compliance with supplementation can be assumed as the strengths of this study. However, there were some limitations as well. We did not have any access to dual-energy X-ray absorptiometry as the gold standard for body composition measurements; therefore, BIA was applied to measure body composition. Further, BIA has been previously validated; so, it was strongly consistent with the results obtained from dual-energy X-ray absorptiometry. Although participants were asked to maintain their regular diets and physical activities, it was not unlikely that they had manipulated their regular life style according to our intervention. Moreover, mitochondrial biogenesis consists of many blood and muscle markers that cannot be assessed because of the financial restrictions. For example, AMPK, calmodulin and PPAR can be assessed as upstream and downstream factors to determine the regulating pathways or muscle biopsies for the fibres alteration assessment.

Conclusion

The present study, for the first time, provided strong evidence showing the effects of HIIT and/or CV on the body composition, performance parameters and mitochondrial biogenesis in the obese women. It seems that HIIT plus CV could reduce body fat percentage, without any synergistic effect of CV and HIIT. Using CV in combination with HIIT also resulted in a marginal

significant increase in PGC-1α level and body water compared with placebo group. There were significant increases in the Bruce MET in HIIT plus CV and HIIT plus placebo compared with placebo group. Beside this, the synergistic effect of CV and HIIT on the Bruce MET increment was found. Furthermore, CV + HIIT and HIIT groups displayed a significant increase in relative VO_{2max} and absolute VO_{2max}, as compared with the placebo; nevertheless, the synergistic effects of CV and HIIT on these parameters were not significant. No significant changes were, however, observed in BMI, FFM, visceral fat, SIRT-1 and FGF-21. These results may, thus, provide new options in the recommended exercise approach for the subjects seeking better performance and body composition. Longer-term clinical trials are also warranted with endpoints associated with mitochondrial adaptation.

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Supplementary material

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