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Research Article

Effect of 8-week metabolic resistance training and Chlorogenic acid supplement on the expression of UCP1: A randomized clinical trial

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Abstract

Background: Obesity and overweight is one of the major public health challenges all around the world. The aim of this study was to evaluate the simultaneous effect of eight weeks of metabolic resistance training (MRT) and Chlorogenic Acid (CGA) supplementation on weight loss, expression level of UCP1in overweight women.

Materials and Methods: The present study was a randomized clinical trial performed in 2022 on a sample of overweight women in Iran. Participants in the study were randomly divided into four groups including combined 8-week course of MRT training and CGA supplementation, 8-week course of MRT, CGA supplement, and the control group. Intervention included three training sessions per week and the duration of each session was 45 minutes. The supplementation arms were also received 400 mg / day CGA extracted from green coffee beans. Expression level of Uncoupling Protein 1 (UCP1) was the main interested outcome that assessed pre and post intervention.

Results: In the MRT exercise group, UCP1 expression increased by 4.3 units on average over the 8-week intervention. The highest increase was observed in participants who received both CGA supplement and MRT exercise where UCP1 increased from 22.5 (1.2) to 28.0 (3.5) over the study period ($P<0.05$). No significant increase was observed in CGA supplementation group.

Conclusion: MRT exercises with and without CGA supplementation stimulated expression level of UCP1 mRNA.

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1. Introduction

Obesity is one of the leading public health challenges worldwide (1). It is estimated that the prevalence of obesity has increased triple times since 1975(2). The number of overweight adults exceeded 1.9 billion in 2016 and 650 million of them were obese(2). Obesity is an independent risk factor for a couple of cancers, diabetes mellitus, cardiovascular diseases, and hypertension and through this pathway has a major contribution to the burden of disease (1, 3).

Several different approaches like exercise training have already been introduced to combat obesity and overweight. According to previous studies, both aerobic and resistance training could lead to favorable outcomes in obese people (4, 5). However, higher efficacy has been shown for metabolic resistance training which is the combination of both aerobic and resistance training (6). The distinctive feature of these exercises is their very small volume, however, it has been shown that the implementation of such exercises leads to an increase in the activity of aerobic and anaerobic enzymes and an increasing amount of both oxidative and glycogenic enzymes, as well (7, 8).

Dietary supplements are another approach to weight reduction and it is illustrated that combined approaches including both dietary supplements and exercise training may bring more benefits (9). There are pieces of evidence regarding the efficacy of Chlorogenic Acid (CGA) in weight reduction in overweight people(10). According to the available evidence, CGA could affect glucose metabolism and fat formation which consequently leads to accelerated weight loss (10, 11).

The possible mechanisms of these interventions have remained almost unclear. It is already shown that practical approaches modulate gene expressions and through this pave increase fat-burning and energy consumption in the cell and the whole body. Uncoupling Protein 1 (UCP1) is the type of protein that contributes to the heating and fat-burning process(12, 13). Therefore, any increase in the expression level of UCP1 can increase the amount of energy consumed by the cell and it is associated with burning reserved fat and consequently weight loss.

In the current, study we aimed to investigate the effect of MRT and CGA supplements on the expression level of UCP1. We also assessed the combined effect of these two approaches in this regard.

2. Materials and Methods

Study design and study participants

We performed an open-label randomized clinical trial on 40 overweight women. The study was carried out on a convenient sample of overweight women referring to a fitness gym in Tehran-Iran in 2022. Inclusion criteria were Body Mass Index (BMI) between 25 and 28 and physical activity in the past six months to maintain health. Not using dietary supplements, medications, underlying diseases related to the investigated variables, and not having any pulmonary diseases were the other inclusion criteria. We excluded cases with no willingness to participate and study participants were allowed to leave the study at any phase.

Intervention

The study participants were randomly assigned into four groups including control groups (no intervention), CGA supplement, MRT exercise, and dual supplement and exercise group who received both CGA supplement and MRT exercise interventions concurrently. The number of training sessions was three a week and the duration of each session was 45 minutes including 10 minutes of warm-up, 30 minutes of metabolic resistance training, and 5 minutes cool down. Details of the exercise intervention are provided in supplementary Table 1. The intensity of the exercise was 60-70 percent of the maximum heart rate of the participants (7). We also used Chlorogenic Acid (CGA) extracted from green coffee as the dietary supplement. Each participant in the supplement group received 400 mg of the green coffee extract on daily basis(14).

Supplementary Table 1: The MRT program for 8-weeks Micro cycle

Complete 1-6 in circuit fashion	Weeks 1-2, 60 seconds rest	Weeks 3-4 45 seconds rest	Weeks 5-8 30 seconds rest
1. Skipping 10 yards down and back	Reps:2 Rounds: 2	Reps:3 Rounds: 2 (3), 3(4)	Reps: 4 Rounds: 3(5-6), 4(7-8)
2. Agility ladder Half ladder, In In Out Out	Reps:2 Rounds: 2	Reps:3 Rounds: 2 (3), 3(4)	Reps: 4 Rounds: 3(5-6), 4(7-8)
3. Modified barbell Hang Clean and Press 15Kg	Reps:5 Rounds: 2	Reps:8 Rounds: 2 (3), 3(4)	Reps: 10 Rounds: 3(5-6), 4(7-8)
4. Medicine Ball, Move to mountain 10 yards, 8Ib med bar or DB	Reps:1 Rounds: 2 5 med balls/DBs	Reps:1 Rounds: 2 (3), 3(4) 6 med balls/DBs	Reps:1 Rounds: 3 (5-6), 4(7-8) 8 med balls/DBs
5. TRX Bodyweight Row	Reps: 5 Rounds:2	Reps:8 Rounds: 2 (3), 3(4)	Reps: 10 Rounds: 3(5-6), 4(7-8)
6. Med Ball Overhead Slams 8-Ib med ball	Reps: 5 Rounds:2	Reps:8 Rounds: 2 (3), 3(4)	Reps: 10 Rounds: 3(5-6), 4(7-8)
7. Finisher: Bike Sprints	Reps: 4(1), 5(2) Workload: 1.0 kg Work/Rest 10 seconds/ 30 seconds	Reps: 5(3), 6(4) Workload: 1.5 kg Work/Rest 10seconds/20seconds	Reps: 5-6(5), 7-8(6) Workload: 2.0 kg Work/Rest 10 seconds/ 15 seconds

Data collection and outcome assessment

Demographic and biometric data including age, weight, height, BMI, medical history, and volume of physical activity was collected for each participant. We also collected data on lipid profile and gene expression using blood samples taken at the baseline and after eight weeks of intervention. Study participants were asked not to have vigorous exercise two in the last two days leading to blood sampling. Blood sampling was performed from the participant's right-hand vein at 8 AM and all study participants were fasting in the last 12 hours. We collected data on HDL, LDL, and triglyceride using a photometric approach. UCP1 was assessed using genomic DNA extraction. We used real-time PCR to determine the UCP1 expression level. All procedures were repeated at the end of the study after an 8-weeks intervention.

Randomization and concealment

We used a stratified balanced block randomization approach for the random allocation of the study participants into four investigated groups. We generated six blocks of 4 using A and B letters (for example, AABB) and numbered them 1 to 6. Then we throw a dice to determine the training intervention status for each participant. We determined the training intervention status for 4 participants in each attempt according to the dice number and its associated AB block. Overall, ten dice throws were performed for all participants. In this stage, we had two groups (20 participants in each group) that were allocated to either training intervention (Group A) or non-intervention (Group B). Then, we repeated the same procedure as the previous step in each group separately to determine whether or not participants would receive nutrition intervention.

The letter A represented nutrition intervention, and the letter B was the marker of no intervention. Finally, we had a two-letter combination of A and B (repeat was allowed) for each study participant to determine the type of intervention that they were supposed to receive. All combinations were numbered 1 to 40 and then received an 8-digits code containing the numbers and letters. We wrote the letter combinations on paper and put them into a sealed envelope. All pockets were given to the research group. After the enrolment of each participant, we announced the envelope code to be opened and repeated the same process until the last participants. The research group was unaware of the following code over the case enrollment period.

Sample size

We calculated the sample size using the comparing two means equation for sample size calculation. In this equation, α was considered as 0.05 and the power study was 80%. We also assumed the effect of the intervention (MRT training) on UCP1 expression level was 2.5 times higher than the control group (15). The sample size of the study was estimated to be 10 people for each arm of the study, and due to the presence of four groups in the study, the final sample size was 40 people.

Statistical analysis

We described continuous variables as mean and standard deviation. Baseline characteristics were compared using One-way ANOVA. We also assessed within-group variability pre and post-study using paired t-test. We also compared post-intervention UCP1 between group variability using One-way ANOVA. We also investigated the effect of each intervention on UCP1 expression level using a multiple linear regression model. Age and other baseline characteristics including BMI, HDL, LDL, TG, and baseline level of UCP1 expression were entered into the model as possible confounders. Then we used a backward approach to generate the best-fitted model. We also drew a change in UCP1 expression versus a change in body weight. The association between these two variables was investigated using simple linear regression. All statistical analysis was performed using Stata software (Ver 17.0, College Station, Texas, USA). P-values <0.05 were considered significant.

3. Results

Overall, the average age of the study participants was 40.4 (5.5) years and there was no statistically significant difference between the study arms (P-value= 0.948). We also found no significant difference in other baseline characteristics including weight, BMI, HDL, LDL, and TG among the compared groups (P-value>0.05) (Table 1).

Table 1: Study participants baseline characteristics by type of intervention

Characteristics	Control	Supplement	Exercise	Exercise/Supp	P-value
Age, year	39.6±4.7	40.6 ±7.0	41.1 ±4.9	40.4 ±5.9	0.948
Weight, Kg	67.1 ±5.2	69.8 ±3.3	69.2 ±5.1	68.3 ±3.5	0.632
BMI, Kg/m^2	26.5 ±1.4	26.6 ±1.3	26.9 ±1.2	26.1 ±1.2	0.555
HDL, mg/dL	65.3 ±6.8	64.1 ±5.4	64.3 ±8.1	63.9 ±9.3	0.977
LDL, mg/dL	99.9 ±19.9	104.0 ±20.3	100.4 ±25.6	103.5 ±22.5	0.990
TG, md/dL	108.7 ±35.8	104.3 ±22.9	108.9 ±15.9	113.1 ±36.1	0.170

- All variables presented as mean and standard deviation.

The average pre-intervention expression level of UCP1 was 22.3 (4.9) in the control group and remained almost constant over the study period (post-study= 22.1±4.2), while in supplement group UCP1 slightly increased from 22.9 (3.4) to 23.6 (5.1), although it was not statistically significant (P-value>0.05). In the MRT exercise group, UCP1 expression increased by 4.3 units on average over the 8-week intervention. The highest increase was observed in participants who received both CGA supplement and MRT exercise where UCP1 increased from 22.5 (1.2) to 28.0 (3.5) over the study period (P-value<0.05) (Table 2).

Table 2: Pre and post-intervention expression of UCP1 by each arms of the study

Group	Pre-intervention	Post intervention	Difference	P-value
Control	22.3 ±4.9	22.1 ±4.2	0.1 ±1.9	0.549
Supplement	22.9 ±3.4	23.6 ±5.1	0.6 ±3.5	0.552
Exercise	22.7 ±4.4	27.0 ±2.6	4.3 ±2.2	<0.001
Ex/Supp	22.5 ±1.2	28.0 ±3.5	5.4 ±3.1	<0.001
P-value	0.982	0.006	0.003	----

We compared the effect of each intervention versus the control group using multiple linear regression and observed that the effect of exercise was 5.0 times higher than the control group (95% CI= 2.5, 7.4). This effect reached 5.5 in the exercise-supplement group and dual intervention led to 5.5 units (95% CI= 3.1, 7.9) average increase in UCP1 expression level in comparison to the control group. The observed association was statistically significant (P-value<0.001). Using the CGA supplement solely has led to a 1.2 unit increase in the average expression level of UCP1. However, the observed change was not statistically significant (P-value= 0.306) (Table 3).

Table 3: Multiple linear regression to investigate the effect of each intervention on expression level of UCP1 adjusted for possible confounders

Group	Regression coefficient	95% CI	P-value
Control	Reference		
Supplement	1.2	-1.1, 3.6	0.306
Exercise	5.0	2.5, 7.4	<0.001
Supp/Exercise	5.5	3.1, 7.9	<0.001

Model is adjusted for age, baseline BMI, HDL, LDL, and UCP1. CI= Confidence Interval

We also investigated the association between change in UCP1 expression level and change in body weight and observed that each unit increase in UCP1 could lead to 1.3 Kg weight loss (95% CI= -1.9, -0.8) that was statistically significant (P-value<0.001) (Table 4). Figure 1 illustrated the association between weight and UCP1 expression in each arm of the study (Figure 1).

Table 4: The association between change in UCP1 expression and weight change in overall and stratified by each arm of the study

Group	Regression coefficient	95% CI	P-value
Control	0.08	-1.2, 1.2	0.987
Supplement	-0.4	-4.4, 3.6	0.808
Exercise	-0.9	-1.6, -0.3	0.010
Supp/Exercise	-1.8	-2.6, -0.9	<0.001
Overall	-1.3	-1.9, -0.8	<0.001

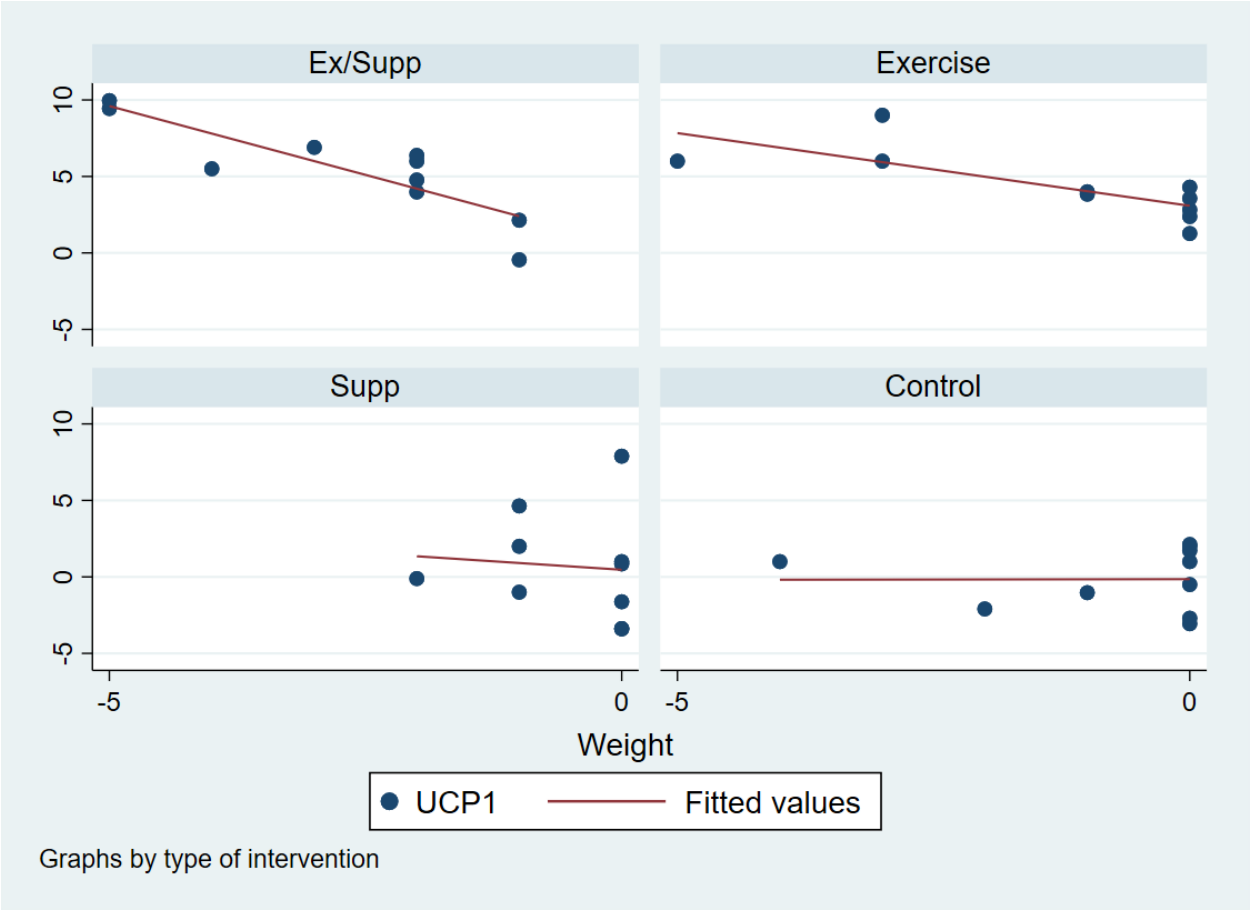

Figure 1: The association between change in UCP1 expression level and weight lost by type of intervention

4. Discussion

The current study aimed to investigate the effect of 8-weeks MRT training and CGA supplement on expression level of UCP1. According to our findings, participation in 8-week MRT training was associated with increased expression level of UCP1. However, such was not observed in CGA supplement group. Moreover, we observed no interaction effect when both interventions were given to the study participants concurrently and change in UCP1 expression was virtually as same as the exercise group. We also found statistically significant inverse association between change in UCP1 level and weight lost. In other word, increase in UCP1 expression was associated with decrease in body weight over the study period.

We observed that MRT exercise either with or without the CGA supplement could increase the expression level of UCP1. Such findings have been previously reported by Daneshyar et al (15). Xu et al have also shown that resistance training could double the expression level of UCP1 in rats which was consistent with our findings(16). Several endocrine mechanisms have been suggested in previous studies for the increasing effect of exercise training on the UCP1 level. According to Daneshyar et al, exercise training increases sympathetic nervous system activity and norepinephrine secretion (17). Exercise-induced norepinephrine binds to β3 adrenergic receptors and therefore induces a signaling pathway leading to UCP1 gene expression in subcutaneous and brown fat cells(17). An increase in thyroid hormones due to contribution in exercise training leading to an increase in volume and activity of the DIO2 enzyme was the alternative mechanism suggested by Daneshyar et al (17).

It is also argued that long-term exercise training increases the secretion of Irisin from muscles and fat tissues that could be considered as a possible pathway to justify the increasing effect of exercise training on UCP1 gene expression(17). Apart from endocrine factors, some proteins like Peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α) might have an effect on exercise-induced UCP1 expression. PGC-1α is a key regulator of energy metabolism(18). According to previous studies, there is a strong correlation between PGC-1α and exercise-induced UCP1 expression (18, 19).

Although more studies have shown that training exercise could increase expression of UCP1, this is still and controversial issue. Flouris et al, in contrast with our findings illustrated that exercise training could not stimulate the expression of UCP1 mRNA(20). Differences in the study design, type and volume of the exercise, and some other confounding factors like temperature, and gender of the participants could justify the observed difference in the previous studies.

We also observed no effect for CGA regarding the stimulation of UCP1 mRNA expression. There was lack of evidence in this regard and the available studies were limited to rats. The study performed by Ye et al reported that CGA was associated with a remarkable increase in the expression of adipogenic and thermogenic genes in Brown Fat Tissues (BAT) (21). Such findings were in contrast with our data. There was also evidence regarding the effect of CGA on the expression of PGC-1α as a modulator of energy metabolism and fat oxidation (22). Huang et al, have also shown that CGA could decrease body weight and obesity through the expression of PPARα which is the opposite of our findings (23).

The study by Sudhakar et al has also shown that CGA has positive effects on the expression of UCP1 and PGC-1α and through this pathway promotes the browning of white adiposities (24). It should be noted that there is a lack of human studies regarding the effect of CGA on the expression of UCP1 and all previous researches were in-vitro or animal-based studies performed on rats. Moreover, we had a small sample size that reduced the power of the study to investigate the association between CGA and UCP1 expression.

The current study is one of the first attempts to investigate the cumulative effect of MRT training and CGA supplementation on UCP1 expression. In this sense, the findings of this research are unique. Conducting the study on a human sample as well as no lost-to follow-up cases in the all intervention and control groups were the main strengths of the current study. However, this study had some limitations that must be considered in interpreting of the results. The impossibility of blinding was the main limitation of the current study, which could bias the findings of the present study. However, in this study, we tried that to avoid contact between the intervention and control groups, and the people of these two groups participated in their exercise programs separately.

Conclusion

According to our findings MRT exercises with and without CGA supplementation stimulated expression level of UCP1 mRNA. There was no such effect for CGA solely. More human studies are required.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval This study was reviewed and approved by Ethics Committee and review board Sport Science Research Institute of Iran (Ethic Code: 95RI-REC-2202-1483 (RS)). The trial was registered at Iranian registry of Clinical Trial irct.ir (IRCT2020051104740).

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: M.T., F.GH., KH.E., SH.S.; Methodology: M.T., F.GH., KH.E., SH.S.; Software: M.T., F.GH., KH.E., SH.S.; Validation: M.T., F.GH., KH.E., SH.S.; Formal analysis: M.T., F.GH., KH.E., SH.S.; Investigation: M.T., SH.S.; Resources: M.T., F.GH., KH.E., SH.S.; Data curation: M.T., KH.E., SH.S.; Writing - original draft: KH.E., SH.S.; Writing - review & editing: M.T., F.GH., SH.S.; Visualization: M.T., F.GH., KH.E.; Supervision: M.T., F.GH., KH.E., SH.S.; Project administration: M.T., F.GH., KH.E., SH.S.; Funding acquisition: M.T., F.GH.

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