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Research Article

The effects of high-intensity functional training with spinach-derived thylakoid supplementation on Irisin and Lipocalin-2 in obese males

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Abstract

Introduction: Obesity is a major cause of mortality in developing countries. Increasing physical activity and improving dietary patterns are effective strategies for its prevention and control. This study examined the effects of a 12-week high-intensity functional training (HIFT) program combined with spinach-derived thylakoid supplementation (T) on circulating lipocalin-2 and irisin levels in obese men.

Methods: Forty-four volunteers (mean age 27.6 ± 8.4 years; mean height 168.4 ± 2.6 cm; mean weight 95.7 ± 3.8 kg; mean BMI 32.6 ± 2.6 kg/m2) were randomly assigned to four equal groups (n = 11 each) including placebo control (C), thylakoid supplement (T), HIFT, and HIFT + thylakoid (HT). After baseline measurements, the HIFT and HT groups completed a 12-week HIFT program based on CrossFit principles (3 sessions/week, total 36 sessions; 60 min/session). Supplement groups consumed 5 g/day T powder; C group received 5 g/day raw corn starch (one sachet, taken 30 minutes before lunch) for the same period. Assessments were performed 48 hours before the intervention and 48 hours after the final training session. Between-group differences were analyzed using analysis of covariance (ANCOVA).

Results: ANCOVA revealed significant between-group effects for lipocalin-2 (P< 0.001) and irisin (P= 0.006). Post hoc pair wise comparisons showed significant increases in irisin and decreases in lipocalin-2 for the T, HIFT and HT groups versus C group (P < 0.001).

Conclusion: Twelve weeks of HIFT and daily T supplementation reduced lipocalin-2 and increased irisin concentrations in obese men, with the greatest improvements observed when training and supplementation were combined.

Keywords:
Functional training, Adipokines, thylakoid, Obese Men

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1. Introduction

Obesity is a leading cause of death in developing countries. It is predicted that by 2035, more than half of the world's population will be overweight or obese (۱). It is estimated that 10.5% of men in Iran were obese in 2010 (۲). Some organs in the body, by secreting certain hormones, exacerbate or reduce obesity-related factors, of which adipose tissue plays a key role by secreting adipokines, muscles by secreting myokines, and the liver by secreting hepatokines. Adipokines, which are secreted by adipose tissue, play a key role in our bodies by affecting obesity, adipose tissue, insulin resistance, and inflammation (۴ ,۳). A chronic, low-grade inflammatory state is created due to increased expression of pro-inflammatory adipokines and decreased expression of anti-inflammatory adipokines, which may contribute to cardiovascular disease as well as systemic metabolic dysfunction in obesity (۵). Examples of novel adipokines that have recently been identified in obesity, metabolic dysfunction, and cardiovascular disease are included the pro-inflammatory lipocalin-2 as a pro-inflammatory and irisin as an anti-inflammatory (۶-۸).

Serum levels of lipocalin 2 are 60% higher in obese individuals (۹). This protein, a member of the lipocalin family, is known as neutrophil gelatinase-associated lipocalin (NGAL). It was first identified in the special granules of human neutrophils and later in other tissues such as liver, kidney, adipocytes and lungs, where it is expressed and secreted by several inflammatory stimuli such as liposaccharides (LPS), tumor necrosis factor alpha (TNF-α) and interleukin-1β (۱۱ ,۱۰). Studies have shown that the LCN2 gene is genetically up-regulated in the adipose tissue and liver of obese animals (۲). Recently, it has been reported that lipocalin 2 is associated with obesity and insulin resistance in rats and humans (۱۲). This protein is significantly expressed by adipocytes in *vivo* and in *vitro*. Lipocalin-2 expression is increased by insulin resistance-promoting factors and is inhibited by thiazolidinediones (۱۳). Circulating lipocalin-2 levels are higher in obese than in lean individuals (۱۱). Lipocalin-2 has also been shown to potentially be involved in obesity-related cardiovascular diseases by regulating inflammatory responses (۱۴). Another adipokine with anti-inflammatory effects is irisin. Irisin, which regulates glucose uptake in skeletal muscle, enhances glucose and fat metabolism, while at the same time reducing hyperlipidemia and hyperglycemia associated with obesity and metabolic syndrome (۱۵). Irisin, which can increase insulin sensitivity, reduces the incidence and severity of type 2 diabetes (۱۶ ,۱۵). One way to control and, to some extent, treat obesity and related diseases is through physical activity. Today, the importance of exercise in reducing inflammation and inflammatory factors has received more attention than ever before (۱۷). According to recommendations from the World Health Organization, the United States Department of Health and Human Services, and other organizations, individuals should engage in at least 150 to 300 minutes (moderate) or 75 to 150 minutes (vigorous) of physical activity per week (۱۸) . One training method to alter body composition and elicit a broad range of metabolic responses is high-intensity functional training (HIFT), which has been proposed as a time-effective approach to improving health in inactive, overweight/obese individuals. HIFT consists of a variety of exercise modalities, such as monotonic aerobic activities (e.g., running, rowing), bodyweight movements (e.g., squats), and weightlifting derivatives (e.g., shoulder presses, dead-lifts) (۱۹).

Unlike HIIT, which tends to focus on single exercise modalities (e.g., running). HIFT emphasizes functional, multi-joint movements that can be modified to suit individual fitness levels and can lead to greater gains in muscle mass than aerobic and resistance training alone (٢٠ ,١٩). Recently, a significant reduction in body fat percentage (~6.5%) was reported after 16 weeks of training in a group of healthy adults (٢١). Furthermore, HIFT has been shown to be an effective strategy for maintaining adherence and enjoyment among sedentary adults (٢٢).

In addition, there is growing interest in the use of phyto-nutrients as complementary therapies for the prevention and management of obesity-related complications (٢٣). Recent studies have shown that thylakoids (T), the chloroplast membranes in green leafy vegetables extracted from spinach, have anti-obesity effects (٢٥ ,٢٤). The beneficial effects of T consumption on obesity and related metabolic disorders, including lowering blood lipids and glucose and improving insulin resistance, have been supported by some evidence (٢٧ ,٢٦). Significant reductions in hunger ratings and cravings for high-sugar foods as well as salty and high-fat foods are the most common effects reported by studies (٢٦-٢٤). In a clinical trial, daily consumption of 5 g of T in the form of a glass of cranberry drink for 3 months in obese women was associated with significant reductions in body weight and waist circumference, as well as decreases in LDL C, fasting glucose (FBG), and fasting insulin (٢٦). Since HIFT is a suitable training model that improves all energy systems, including phosphagen, cyclolysis, and beta-oxidation, and T supplementation is also a complete nutritional supplement containing essential vitamins and proteins, as well as being rich in antioxidants, and can reduce inflammation caused by obesity, it is therefore important to address the effective mechanisms of HIFT as a complete and new training model and T supplementation as a supplement rich in antioxidants on these adipokines. Therefore, the present study investigated the effect of HIFT and T supplementation on plasma irisin and lipocalin-2 levels in obese men.

2. Materials and Methods

Since the subjects of the study were obese men and were studied in a 12-week research project, the present study was an applied and semi-experimental type. The participants in this study were obese male volunteers who were selected through a call in public and administrative centers. The conditions for entering the study were no drug or alcohol addiction, no history of regular exercise for at least 6 months, no history of kidney, liver, cardiovascular disease, diabetes, and having conditions (BMI = 30) and no physical injury or problem for the subjects, and they were included in the study after being examined by a cardiologist. Before participating in the study, all steps and procedures were explained to them, and after full information and completing a medical questionnaire, written consent was obtained from them. Finally 44 people with an age range of 23-32 years were selected. In the first session, height and weight were taken from all subjects and all stages of the research and how to perform the exercise program were shown to the subjects. Then, the subjects were divided homogeneously into 4 groups based on body mass index including 1) control-placebo (H), 2) T, 3) HIFT and 4) HIFT + T (HT). Then, the T, HIFT and HT groups completed their research period based on the determined protocol, which is described below.

However, the C group performed their daily activities for 12 weeks and they were prohibited from participating in regular activities. All ethical principles were observed during the training period and the subjects were allowed to withdraw from the research at any time during the training period. This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved in December 2022 by the Iranian Clinical Trials Registry System (IRCTID: IRCT20151228025732N77) and the Research and Ethics Committee of Islamic Azad University, Damghan Branch (Ethics Code: IR.IAU.DAMGHAN.REC.1401.034).

The HIFT program consisted of 36 (60-minute) sessions, using CrossFit trainings. The first two sessions of the training program were simply to familiarize with the common movements (squat, dead-lift, press, jerk, barbell, dumbbell movement, medicine ball clean, barbell pull-up movement, and kettle-bell swing movement) in HIFT (Table 1) (۲۸).

Table 1. HIFT- based training schedule

Bodyweight exercises	Aerobic exercises	Weighted exercises
Squats without weights	Running	Dead-lifts
Pull-ups	Cycling	Cleans
Push-ups	Rowing	Presses
Dips	ropes	Snatch
Swimming or push-ups on the shoulder		Clean and jerk
Rope climbs		Exercises using a medicine ball
Muscle ups		Kettle-bell swings
Lumbar fillets		Exercises with dumbbells
Pull-ups		Barbell
Types of jumps		
Lunges		

From the third day, each HIFT session included 10-15 stretching and warm-up movements, 10-20 minutes of instruction, technique and movement practice, and 5-30 minutes of workout of day (WOD) at maximum intensity according to the individual's personal ability and fitness level. The daily exercise (WOD) consisted of aerobic exercise (e.g., running, skipping), bodyweight exercises (e.g., pull-ups, squats), and weightlifting (e.g., front squats, kettle-bell swings), which were continuously varied using the CrossFit training pattern. The single (e.g., M), double (e.g., G, M), and triple (e.g., M, G, W) training sessions were performed according to Table 1, such that the training program consisted of three days of training and one day of rest, and the first day of WOD training included one type of activity, the second day of WOD training included two types of activities, and the third day of WOD training included three types of activities, and the fourth day of rest. The conditions of all weights and exercises were individually described and customized for each participant. Each day, the duration of each workout, the total rounds and repetitions completed for each exercise, the weights used, and any necessary modifications to the workout program were recorded for each participant(۲۹ ,۲۲).

In single-activity sessions; only one type of movement or activity was performed (M: performing a long-distance, slow-paced activity) - G: performing a heavy skill - W: performing a heavy weight, low-repetition movement). On days G and W, recovery was long-term and complete. In double-activity sessions; the two movements were performed alternately (activity-rest) with 3 to 5 rounds and at moderate to maximum intensity. The first round was hard but doable, and subsequent rounds were performed at a lower intensity. In triple-activity sessions; the three activities were performed for 20 minutes and the number of repetitions performed during this time was recorded, and an attempt was made to perform the most repetitions.

Supplement

Fresh spinach leaves (Spinacia oleracea) were used to prepare T membranes according to previously published protocols. The scientific name of the collected specimen is Spinacia oleracea L. belonging to the Oleracea family. The T supplement used in this study was prepared according to the method described by Emerk *et al.,*. After removing the stems and veins of spinach, the fresh spinach leaves were soaked in cold water and washed. Spinach leaves (1000 g) were homogenized with 1250 ml of water in a blender and filtered through a four-layer monodor polyester mesh (20 μm). The obtained filtrate was diluted 10 times with distilled water and its pH was adjusted to 4.7 with hydrochloric acid (HCl). pH 4.7 is the isoelectric point of Ts and maximum sedimentation occurred at this pH. After 4 hours of cold storage (-4°C), a green precipitate with a clear, slightly yellow supernatant was obtained. The supernatant was removed and the green precipitate was collected from the purified Ts at pH 4.7 and washed by repeated centrifugation in water. The sedimentation was repeated at the same pH. The washed Ts were collected and after adjusting the desired pH (pH=7), the final precipitates were freeze-dried to obtain green T powder.

The placebo consisted of cornstarch, which was edible green and flavored with kiwi essential oil, like T powder. Cornstarch, a white, tasteless, harmless, non-toxic, non-irritating, odorless and allergenic powder, is the most widely used ingredient. It is used in the food and pharmaceutical industries as an inert (inactive) material without therapeutic effect. Cornstarch was also changed to a green powder with a kiwi odor, just like the T extracted from spinach. Therefore, green T powder or kiwi-flavored placebo were made with the same appearance (shape, size and color).

They were then packaged in identical bags with each bag containing 5 g of T or 5 g of cornstarch powder. The bags were coded and distributed to the participants. During consumption, the contents of the bags were dissolved in a glass of water and consumed 30 minutes before lunch(۳۰).

Blood sampling

The first blood sample gathered 48 hours before and the second blood sample gathered 48 hours after the twelve-week training period from the right brachial vein of the subjects. The blood samples transferred to special test tubes for plasma preparation (tubes containing EDETA) and centrifuged for 10 minutes at 3000 rpm. The resulting plasma stored at -70 degrees Celsius. It should be noted that all stages of the test performed under the same and standard conditions from 8- 10 in the morning. Lipocalin-2 was measured using an ELISA device and kit from Elabscience Biotechnology, catalog number: E-EL-H0096. Irisin was also measured using an ELISA device and kit from Phoenix Pharmaceuticals Inc, catalog number: EK 067-16.

Statistical analysis

The kolomogorov- Smirnov test used for measure the normal distribution of data. The paired sample t-test was used to compare the changes in the pre-test and post-test in each group. The analysis of covariance (ANCOVA) test with Bonferroni post- hoc test were used to compare between groups. All data were expressed as mean ± standard deviation. All analyses were performed using SPSS version 22 statistical software ($P<0.05$).

3. Results

The distribution of data was examined using the kolomogorov- Smirnov test, which showed that the research data were normally distributed ($P<0.05$). A significant difference between groups (C, T, HIFT, and HT groups) was observed for lipocalin- 2 levels using the analysis of covariance test ($P<0.001$).

Comparison of research groups using the Bonferroni post- hoc test showed that there was a significant difference between the T group and the C ($P=0.015$), the HIFT group and C group ($P<0.001$), and also between the HT group and the C ($P<0.001$) for plasma lipocalin-2 levels. However, no significant difference was observed between the other research groups ($P<0.05$). Also, the results of the intra-group test showed that a significant decrease was observed in the T ($P<0.001$), HIFT ($P<0.001$) and HT ($P<0.001$) groups compared to the pre-test (Figure 1).

Significant between-group differences (C, T, HIFT and HT groups) were observed for irisin levels by analysis of covariance test ($P=0.006$). Comparison of study groups using Bonferroni post- hoc test showed that there was a significant difference between T and C group ($P=0.011$), HIFT group ($P=0.008$) and also between HT and C group ($P=0.020$) for plasma irisin levels. However, no significant difference was observed between other study groups ($P<0.05$). Also, the results of the intra-group test showed that a significant increase was observed in the T ($P=0.021$), HIFT ($P<0.001$), and HT ($P<0.001$) groups compared to the pre-test (Figure 2).

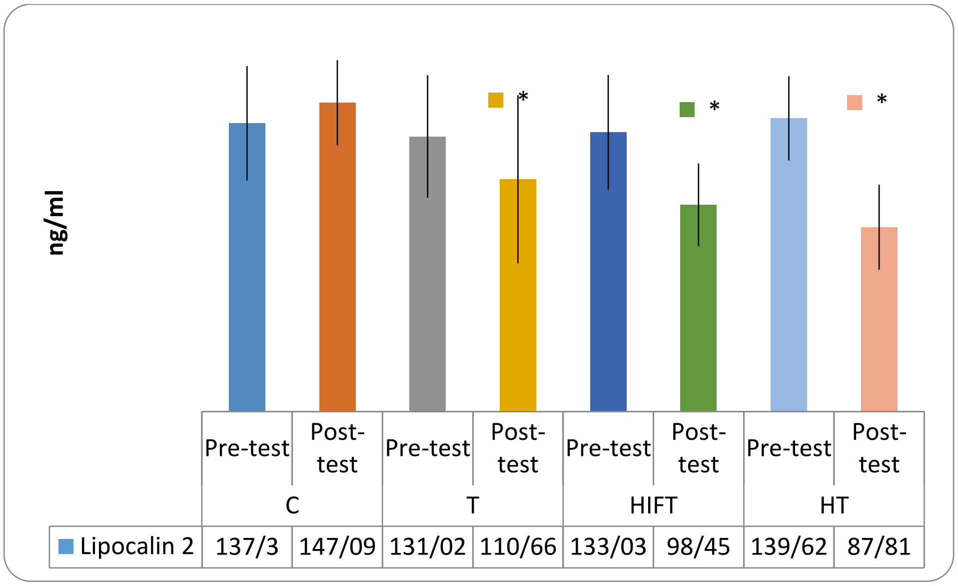

Figure 1. Lipocalin 2 levels. * Significant decrease compared to C group

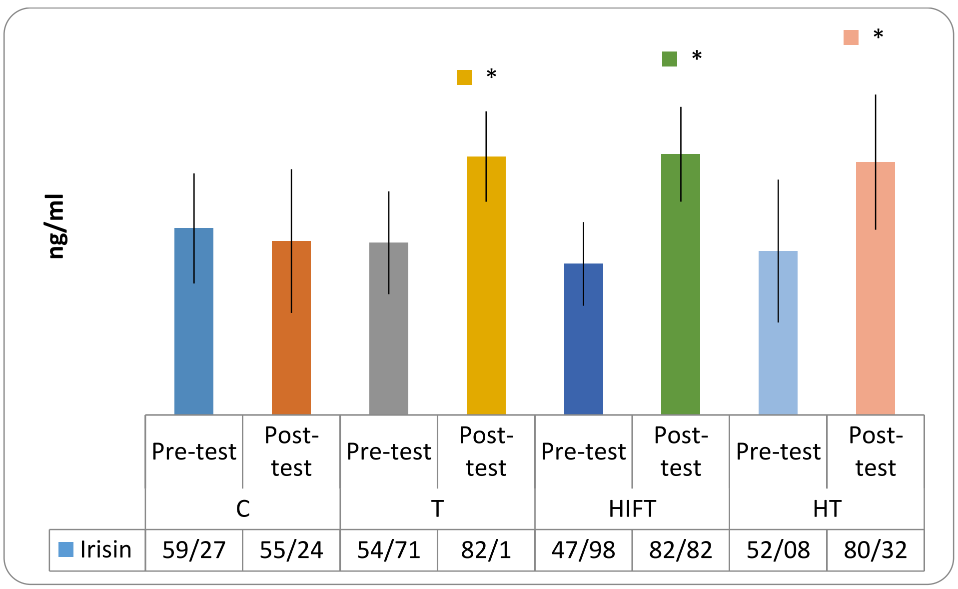

Figure 2. Irisin levels. * Significant increase compared to C group

4. Discussion

The aim of the present study was to determine the effect of 12 weeks of HIFT combined with T supplementation on lipocalin 2 and irisin levels in obese men. The present results showed that HIFT alone and in combination with T supplementation played an important role in modulating circulating adipokines, and although T alone also had an effect on some adipokines, in combination with HIFT it had a significant role in modulating circulating adipokines. Exercise is an effective anti-inflammatory intervention to combat the consequences of inflammation due to its role in increasing and decreasing anti-inflammatory and inflammatory adipokines, respectively (۳۱). HIFT is a new type of training program that researchers have reported to have anti-inflammatory effects similar to traditional resistance training in young obese individuals (۳۲). Lipocalin 2 was one of the adipokines examined in the present study, which showed a significant decrease with HIFT and HT, as well as T consumption alone, compared to the C group. A decrease of 15.33, 25.99, and 37.1% of lipocalin 2 was observed in the T, HIFT, and HT groups, respectively. Researchers have suggested that increased lipocalin- 2 levels in obesity and type 2 diabetes may be a compensatory mechanism to counteract the metabolic disorders of obesity through its anorexigenic action and increased beta-cell function (۳۳). Some researchers have also suggested that due to the strong association of lipocalin- 2 with obesity, chronic inflammation (especially the positive correlation with CRP), and obesity- related disorders, targeting and modulating its levels could play a major role in combating obesity-related disorders, especially the inflammatory pathways associated with it (۱۱). The present results, confirming these points, showed that HIFT could be an effective intervention to reduce lipocalin- 2 levels, thereby helping to improve insulin resistance and counteract other disorders caused by increased lipocalin- 2. Despite the present results, conflicting results have been reported regarding changes in lipocalin- 2 with exercise, and researchers have shown a decrease (۳٤) and no change(۳٥) of this adipokine with exercise. Researchers have introduced moderate-intensity continuous exercise and HIIT as effective interventions for down-regulating lipocalin- 2 after 12 weeks in obese women and considered the reduction in body fat percentage and BMI as possible mechanisms for the reduction in lipocalin- 2. In addition, as in the present study, the reduction in lipocalin- 2 was associated with a decrease in insulin resistance and an improvement in the lipid profile (۳۴). One of the main reasons for the contradiction between the above findings and the present results regarding the lack of a simultaneous reduction in body fat percentage and BMI with lipocalin- 2 may be the different types of samples studied, such that in the above study, young men with normal weight were studied, and in the present study, obese men were studied. Naturally, due to the obesity of the subjects studied in the present study, greater changes in body fat mass and body weight should be expected with exercise training. Of course, the duration of the exercise period in the present study was also four weeks longer than in the above study and its type was also different. These results indicate the modulating role of exercise on lipocalin- 2 independent of changes in body composition and even other inflammatory mediators.

Irisin is one of the known adipo- myokines that was investigated in the present study and the results showed a significant increase in Irisin with T consumption, HFT and HT compared to the C group. By calculating the percentage of changes, it was determined that irisin increased by 50.06, 72.61 and 54.22% in the T, HIFT, and HT groups, respectively. Skeletal muscle, as the largest endocrine organ of the body, secretes hormones called myokines into the bloodstream. Although muscle tissue is the main site for irisin secretion, irisin is also known as an adipokine that is secreted by visceral adipose tissue, especially subcutaneous adipose tissue, and exercise can stimulate the secretion of FNDC-5 in visceral and subcutaneous adipose tissue (۳۶).

In overweight/obese human models, irisin has also been shown to be increased compared to overweight individuals, although this increase may vary depending on the race and ethnicity of the subjects. Despite confirmation of increased irisin in Africa, this increase has not been reported in Asia, Europe, or the Americas, suggesting that increased irisin levels in obesity are an adaptive compensatory response to the metabolic disorder caused by obesity, such as reduced insulin levels or irisin resistance (۳۷).

Irisin has been shown to have anti-inflammatory effects on adipocytes and macrophages. Irisin significantly alters macrophage activity, improves their ability to phagocytosis, and reduces the severity of processes associated with ROS production, which may indicate the potential anti-inflammatory properties of irisin (۳۸). Subsequent studies have shown that irisin exerts its anti-inflammatory properties through down-regulation of downstream pathways such as TLR4/MyD88 (۳۹). Macrophage infiltration into adipose tissue is associated with obesity, which causes a shift in the macrophage phenotype from the anti-inflammatory M2 to the pro-inflammatory M1 (۴۰). Irisin can reverse this process by stimulating macrophage polarization from M1 to M2 (۴۱) . On the other hand, irisin can reduce the expression of inflammatory cytokines such as TNF-α, IL-6, MCP-1 and MIP-1α and, conversely, increase the expression of IL-10 in human subcutaneous and visceral adipose tissue, although the anti-inflammatory effects of irisin have also been shown in other body tissues (٤٢). In summary, it has been suggested that the anti-inflammatory effects of irisin are exerted by modulating the expression and production of cytokines, affecting transcription factors such as MAPK and NF-κB, or reducing the production of reactive oxygen species (٤٢), and it is likely that the positive effects of irisin are exerted in part by these anti-inflammatory effects. The present results showed that HIFT is a sufficient stimulus to increase circulating irisin. Consistent with the present results, in a review study, researchers stated that exercise in various forms of aerobic, resistance, combined, and HIIT leads to an increase in irisin, which results in thermogenesis and ultimately body weight loss in obese individuals (٤٣). Increased expression of PGC-1α, which can stimulate FNDC-5 expression, is one of the major mechanisms for stimulating irisin secretion in response to exercise (٤٤).

In the present study, in addition to HIFT, the effect of T consumption alone and in combination with HIFT was also investigated. T consumption with HFT resulted in significant improvements in the levels of all adipo-myokines, similar to the HFT group, and except for irisin, the greatest changes in variables were observed in the HT group. Importantly, T alone was also able to stimulate a significant decrease in lipocalin- 2 and an increase in irisin levels. However, HT did not have an additive or synergistic effect on the variables compared to HIFT alone, and despite the observation of an increased effect of HIFT on adipo-myokines, no significant difference was observed between the HIFT and HT groups. T has numerous positive effects on obesity, and the use of this supplement can help manage obesity and its associated disorders. In this regard, T have been recognized as an effective supplement for increasing anti-obesity activity (satiety induction, increased lipolysis), improving glucose and insulin metabolism. Anti- inflammatory, and also antioxidant effects of Ts is mainly due to their galactolipid components (٤٥). Antioxidant compounds of Ts such as chlorophylls, flavonoids, and carotenoids such as zeaxanthin and lutein may be beneficial in reducing oxidative stress and increasing antioxidant properties by scavenging ROS.

In addition to antioxidant effects, chlorophyll derivatives present in Ts can also affect PPARs, which are involved in the regulation of metabolic pathways such as insulin sensitivity, glucose homeostasis, lipid metabolism, fatty acid oxidation, and anti-inflammatory responses. Therefore, activation of PPARs plays a major role in reducing diseases caused by overweight/obesity, such as dyslipidemia (۴۶). The appetite-suppressing and fat- and glucose-lowering properties of Ts have been demonstrated in previous studies. The satiety-promoting effects of Ts may be due, in part, to reduced fat digestion by inhibiting pancreatic lipase/colipase activity, which induces the secretion of hormones such as cholecystokinin and glucagon-like peptide-1 (GLP-1) that signal satiety (۴۷). Indeed, T supplementation reduces body fat and fat cell size by binding to dietary fat and increasing its excretion in the feces, thereby reducing the absorption of dietary fat (۴۸). Other mechanisms by which Ts induce satiety and body weight loss include decreased levels of ghrelin (the hunger hormone) and increased levels of leptin (the satiety hormone), which ultimately induce body weight loss and fat mass loss by increasing fat oxidation (۴۷). In fact, based on the above-mentioned cases and the present findings, some of the positive effects of Ts, including improving the adipo- myokine profile, should be associated with anti-obesity effects (reducing fat mass and body weight), anti-appetite effects, anti-inflammatory and antioxidant effects, as well as its role in improving insulin resistance (increasing glucose metabolism). Despite the above-mentioned cases, due to the limited resources on the simultaneous effect of Ts and exercise in various forms, identifying the mechanism of the positive effects of Ts, including its role in modulating adipokines levels, especially in combination with exercise, requires further investigation, and future research can largely answer the ambiguities in this field. Several important limitations of this study should be noted. First, the selection of only men was made to enhance internal validity; however, this decision reduces the generalizability of the results to the female population, as hormonal fluctuations in women can alter adipokines responses and the effects of exercise or supplement interventions. Therefore, future research needs to either include women or provide separate analyses by gender. Second, although 3-day food records were collected, careful control of caloric intake and macronutrient composition was not achieved over the 12-week period, and plasma glucose and insulin concentrations were not measured. This limits the mechanistic interpretation of the changes in adipokines and adipokines; in future studies, it is necessary to control or standardize the dietary strategy and to assess markers related to glucose-insulin metabolism. Third, the relatively small sample size (about 11 subjects per group) may reduce the statistical power required to detect smaller effects. Also, the lack of measurement of oxidative stress indices, insulin signaling pathways, and hepatokines has prevented a complete elucidation of the mechanisms underlying the changes in adipokines. Therefore, it is suggested that future studies be designed with larger samples, longer follow-up, and a broader biomarker panel to investigate possible mechanisms.

۵. Conclusion

The present results support the positive role of functional training alone and in combination with T consumption, and that T alone can also affect adipo-myokine levels, such that it reduces lipocalin-2 and increases irisin. Based on the present results, it can be concluded that the positive effects of the present study's interventions in obese individuals are partly dependent on the modulation of adipo-myokine-related signaling pathways, and given the greater but non-significant effect of T+ HIFT compared to HIFT alone, higher doses of T consumption or longer periods of intervention may be needed to observe the synergistic effect of T with exercise. Nevertheless, due to the positive effect observed for T and its role in increasing the effectiveness of exercise, albeit non-significantly, its inclusion in the diet of obese individuals under the supervision of a nutritionist may be considered.

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Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Informed consent Informed consent was obtained from all participants.

Author contributions

Conceptualization: L.V, S.A.H, H.P, M.A.A ; Methodology: L.V, S.A.H, H.P, M.A.A ; Software: L.V, S.A.H, H.P, M.A.A ; Validation: L.V, S.A.H, H.P, M.A.A ; Formal analysis: L.V, S.A.H, H.P, M.A.A ; Investigation: L.V, S.A.H, H.P, M.A.A ; Resources: L.V, S.A.H, H.P, M.A.A ; Data curation: L.V, S.A.H, H.P, M.A.A ; Writing - original draft: L.V, S.A.H, H.P, M.A.A ; Writing - review & editing: L.V, S.A.H, H.P, M.A.A ; Visualization: L.V, S.A.H, H.P, M.A.A ; Supervision: L.V, S.A.H, H.P, M.A.A ; Project administration: L.V, S.A.H, H.P, M.A.A ; Funding acquisition: L.V, S.A.H, H.P, M.A.A.

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