



## The Effect of High-Intensity Functional Training with Thylakoid Supplementation on Anti-Inflammatory Indicators in Obese Men

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### ABSTRACT

**Background:** Obesity is associated with a chronic low-grade inflammatory state related with vascular dysfunction, thrombotic disorders, multiple organ damages, and metabolic dysfunction. To date, no study has examined the effects of different exercise protocols on the profile of inflammatory factors in the body and the use of dietary supplements. The objective of this study was to investigate the effect of 12 weeks of high-intensity functional training (HIFT) with and without thylakoid supplementation on IL-10 and semaphorin-3E levels in obese people. **Methods:** 44 obese men were randomly assigned to one of four groups: control (C), thylakoid supplementation (T), high-intensity functional training (H), and a combination of supplementation and exercise (HT). The exercise program was performed according to the HIFT protocol for 12 weeks. The thylakoid supplement was extracted from spinach leaves and administered to individuals in the supplement groups. Body mass index (BMI) and biomarkers related to inflammation were measured in the participants' blood samples at the before and after the intervention. **Results:** In these three groups, H, T, and HT, the levels of the inflammatory factors IL-10, and Semaphorin-3E were compared. In all the three groups, the levels of the anti-inflammatory factor Semaphorin-3E and IL-10 changed significantly ( $P < 0.001$  vs. C). The level of Semaphorin-3E exhibited a statistically significant decline in the blood of the H and HT groups who underwent exercise in comparison to the C group ( $P < 0.01$ ) and a significant difference was observed between the exercise training groups, H and HT, and T group ( $P < 0.001$ ). **Conclusion:** The results of the present study demonstrated that HIFT exercise protocol and the use of thylakoid supplements can reduce systemic inflammatory indicators in obese men. This reduction was observed through a synergistic effect. Consequently, the combination of HIFT exercise and thylakoid supplements represents an effective approach to reducing inflammation in individuals with obesity.

### Introduction

Obesity is a chronic disease that has become a major concern in recent years, as it can have negative impacts on health, leading to increased morbidity and mortality (Popovicu *et al.*, 2023). Obesity is classified as a national epidemic, with

one in three adults and one in six children in the United States of America affected by the condition (Tiwari and Balasundaram, 2021). Several countries worldwide have observed a significant increase in the prevalence of obesity over the past

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three decades, likely due to urbanization, a sedentary lifestyle, and an increase in the consumption of high-calorie processed foods (Tiwari and Balasundaram, 2021). The conditions are caused by excessive fat in the body which results in high risk for several diseases. Body Mass Index (BMI) is used as a screening tool (Boachie *et al.*, 2022). Increased BMI is associated with a higher prevalence of health complications such as cancers, cardiovascular (CVDs) and endocrine diseases, musculoskeletal, and respiratory disorders (Boachie *et al.*, 2022). Given its multifaceted nature, the management of obesity necessitates a comprehensive approach (Atakan *et al.*, 2021). Although pharmacotherapy and bariatric surgery are indicated for severe obesity, diet, exercise, and cognitive behavioral therapy are the primary strategies for the lifelong management of obesity (Atakan *et al.*, 2021). Kheniser *et al.* stated that two years of lifestyle interventions could facilitate a 5% weight loss and that although a weight regain occurs, both diet and exercise interventions have substantial effects on obesity-associated comorbidities and adipose tissue remodeling (Atakan *et al.*, 2021).

The close relationship between inflammation and obesity is further supported by the striking decrease in circulating pro-inflammatory markers following bariatric surgery, which results in important weight loss and dramatic improvements in metabolic functions (Kawai *et al.*, 2021). Obesity is currently conceptualized as a pro-inflammatory state, characterized by an expansion in the outflow of inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ), in addition to the expanded emission of leptin (Battineni *et al.*, 2021). The inflammation of the adipose tissue is characterized by the infiltration of activated M1 macrophages, leading to the production of reactive oxygen species (ROS) and release of pro-inflammatory cytokines, such as IL-6 and TNF- $\alpha$  (Zatterale *et al.*, 2020). Furthermore, chronic inflammation, as opposed to acute inflammation, which is a required and appropriate response to an infection or injury, can lead to the onset and progression of various conditions, such as

diabetes, heart disease, neurological conditions, and cancer (Nigam *et al.*, 2023). Macrophages represent the most prevalent innate immune cells to infiltrate and accumulate within the adipose tissue of obese individuals, comprising up to 40% of all adipose tissue cells in obesity (Henein *et al.*, 2022).

Physical exercise has been demonstrated to improve vascular structure and endothelial function, lower blood pressure and lipid levels, improve glycemic control, and reduce chronic inflammation and BMI. Individuals to overweight often demonstrate motivation to engage in exercise due to body dissatisfaction (Al-Mhanna *et al.*, 2023). It is evident that various forms of exercise have clinically positive effects on populations with obesity, even in the absence of weight loss (Al-Mhanna *et al.*, 2023). Moreover, diet interventions alone can provide T2DM patients with anti-inflammatory benefits but also aerobic exercise alone provokes favorable changes in inflammatory markers in individuals with T2DM and/or obesity (Al-Mhanna *et al.*, 2023).

Recently, Feito *et al.* reported significant reductions in body fat percentage (~6.5%) after 12 weeks of training among a group of healthy adults. Additionally, Heinrich *et al.* showed that HIFT (high-intensity functional training) was an effective strategy for maintaining adherence and enjoyment among sedentary adults. Thus, it seems HIFT may be a potentially beneficial strategy for combating obesity (Feito *et al.*, 2019).

Interleukin-10 (IL-10) is a pleomorphic cytokine with diverse phenotypic effects. Initially discovered as a product of T helper 2 cells that inhibited T helper 1 cell activation, it is now known to be produced by almost all species of activated immune cells, including B cells, mast cells, granulocytes (e.g., neutrophils, basophils, eosinophils), macrophages, dendritic cells, and multiple T cell subsets (Steen *et al.*, 2020). Furthermore, the anti-inflammatory actions of IL-10 can be diminished following exercise training and in the context of chronic inflammatory states such as type 2 diabetes (T2D), irrespective of the concentration of IL-10 in the circulation (Islam *et al.*, 2021).

Moreover, recent studies have investigated the potential synergistic effects of dietary supplements, such as thylakoids derived from green plants, in enhancing the outcomes of exercise interventions. Thylakoids, specialized membrane structures found in chloroplasts, have gained attention for their role in modulating appetite and metabolism. Previous research has demonstrated that these supplements are capable of prolonging satiety, reducing food intake, and improving markers of metabolic health (Saeidi *et al.*, 2023). By studying the existing literature, so far, the effectiveness trial has not addressed the combined effect of HIFT and thylakoid supplementation on anti-inflammatory indicators in obese people.

This article examines the intersection of high-intensity functional training and thylakoid supplementation in the context of reducing inflammation in obese individuals, with a particular focus on its effects on anti-inflammatory indicators in obese men. By examining the existing literature and recent studies, the authors' aim is to shed light on the potential of this combined intervention to alleviate inflammation and improve metabolic health in this population. This study evaluates the effect of 12 weeks of high-intensity functional training (HIFT) with and without thylakoid supplementation on IL-10 and semaphorin-3E levels in obese people.

## Materials and Methods

### Study design

The current research is a semi-experimental type with a pre-test-post-test design. It was conducted in two stages before and after the intervention, and a placebo was used in the field and laboratory to control for external variables.

### Participants

The statistical sample (according to the sample size determination formula) included 44 obese men ( $BMI \geq 30 \text{ kg/m}^2$ ) in the age range of 23 to 32. The samples were selected through a call in administrative and public centers. All experimental procedures were conducted in accordance with the principles of voluntary participation, informed consent, and ethical review. The participants were

informed of the nature of the study and their rights as research participants. They were also informed of their right to withdraw from the study at any time during the training period.

### Inclusion and exclusion criteria

The participant should not be suffering from any chronic diseases, as indicated by the medical history questionnaire (Cardiovascular diseases and diabetes or any injury or problem that limits them from participating in physical activity). Furthermore, the participant should not use any food supplements, smoke, drink alcohol, or undergo drug treatment. Additionally, the participant should not be on a low-calorie diets in the past six months and should not be doing regular daily sports over the past two years. Finally, the ratio of waist circumference to height should be above 0.5, which was one of the criteria for entering this study.

The criteria for exiting the research included the absence of more than three sessions in the exercise program, the occurrence of an accident, a physical injury, or the occurrence of any factor that affected the participants' ability to participate effectively in the training sessions.

### Intervention

When the researcher was ensured of the appropriateness of the volunteer to enter the study according to all entry and non-entry criteria, the candidate, based on the predetermined random plan, was in one of the four groups of intervention.

According to the established schedule, the participants went to the laboratory to complete the personal information, health questionnaire, and physical activity questionnaire, and to check the anthropometric indicators. Also, fasting blood samples of 7 ml were taken from the participants 48 hours before the start of the training protocol and taking the thylakoid supplement and 48 hours after the last training session and taking the supplement to measure inflammatory indicators. After the run-in period, using permuted block randomization, volunteers were randomized in a 1:1 ratio into four study arms.

In this randomized controlled trial, 44 obese

men were recruited and randomly divided into the following groups based on individual characteristics (n=11):

1- Control group (C): The participants in this group were required to refrain from regular sports activities and take a placebo for 12 weeks.

2- Supplemental group (T): The participants in this group consumed 5 grams of thylakoids, which were dissolved in a glass of water 30 minutes before lunch. They did not engage in any exercise during this period. The 12-week intervention duration and a dose of 5 g/day thylakoid were selected based on previously reported beneficial effects of thylakoid supplementation on obesity status and related metabolic profiles in participants who received thylakoid supplements for 12 weeks (Pourteymour Fard Tabrizi *et al.*, 2021).

3- HIFT group (H): The participants in this group engaged in HIFT exercise and took a placebo for 12 weeks.

4- HIFT+supplement group (HT): The participants in this group received thylakoid supplements in addition to the prescribed exercise regimen. They were provided with packages containing thylakoid supplements. In addition to the exercise regimen, the participants were instructed to dissolve the contents of the bags containing the thylakoid supplement in a glass of water and to consume it 30 minutes before lunch.

#### **HIFT exercise protocol**

For 12 weeks, participants were subjected to an HIFT exercise regimen, as detailed by Feito *et al.* in their publication (Feito *et al.*, 2018). The CrossFit program was utilized as the HIFT program. The initial two sessions of the training program were designed to familiarize participants with the common movements included in the program, including squatting, deadlifting, pressing, jerking, barbell movements, dumbbell movements, medicine ball clean movements, pull-up barfix movements, and kettlebell swing movements. The third day of training began with a 10-15 minute stretching and warm-up period, followed by 10-20 minutes of training and training techniques, and concluded with 20-60 minutes of daily exercise

(WOD). This was performed at a very high intensity and according to the individual's ability. The primary training components of aerobic activities (e.g., running, jumping rope), body weight activities (e.g., Traction Barfix, Scott), and weight lifting (e.g., front squats, kettlebell twists) were incorporated into the CrossFit exercises. These exercises were performed in single, double, and triple sets for time, repetitions, or weight. The selected movements from **Table 1** were utilized. The training process in both experimental groups was divided into three stages: warming up, main training, and cooling down. The functional exercises selected by HIFT group and HIFT supplement were performed in each training session in a circular manner and according to the principle of overload. The protocol was implemented over the course of four weeks, with each week comprising a different duration and intensity. The first week involved 20-30 minutes of exercise at an intensity of 40 to 50% of the maximum heart rate, the second week comprised 40 minutes of exercise at a power of 50 to 60% of the maximum heart rate, the third week involved 50 minutes of exercise at a power of 50 to 60% of the maximum heart rate, the fourth week comprised 50 minutes of exercise at a power of 60 to 70% of the maximum heart rate, and finally, the last eight weeks involved 60 minutes of exercise at an intensity of 60 to 70% of the maximum heart rate.

The training sessions were structured according to a schedule comprising one movement (M), two alternating movements (G and M), and three 20-minute activities (M, G, and W). In this program, M is an activity with a long distance and slow speed, G is a heavy skill, and W is a movement with heavy weight and low repetitions. The training program included three days of training and one day of rest. On the first day of WOD training, one type of activity was performed. On the second day of WOD training, two types of activities were conducted. On the third day, three types of activities were completed, and on the fourth day, rest was observed. On each day of the program, the duration of each workout, the total number of rounds and repetitions

completed for each movement, the weights used, and any required changes to the workout program were recorded for each participant. The intensity of the exercise was calculated using Karvonen formula (Yabe *et al.*, 2021). The target heart rate

was determined according to the following formula (Yabe *et al.*, 2021):

$$\text{THR}_{\text{Karvonen}} = (\text{HR}_{\text{peak}} - \text{HR}_{\text{rest}}) \times k + \text{HR}_{\text{rest}}$$

$$\text{Predicted HR}_{\text{peak}} = 220 - \text{age}$$

**Table 1.** Movements used in the high-intensity functional training (HIIFT) protocol.

Movements using body weight	Aerobic activity	Movements with weights
Bodyweight squat	Run	Deadlift
Pull-up (Barfix)	Riding bike	Clean
Push-ups	Rowing	Chest press
Dip	Skipping rope	Snatch
Swimming		Clean and jerk
Rope climbing		Movements using medicine ball
Burpee		Kettlebell Swings
Loin fillets		Dumbbell movements
Sit-ups		Barbell
The types of jumps		Goblet squat
Lunge		

#### Thylakoid supplement preparation

Fresh leaves of baby spinach (*Spinacia oleracea*) were used to prepare thylakoid membranes according to the method described by Emek *et al.* (Pourteymour Fard Tabrizi *et al.*, 2021). Fresh spinach was collected from Tabriz, East Azarbaijan Province, Iran in the spring of 2023. The specimens of *Spinacia oleracea* L. (belonging to the *Oleracea* family). The stems and veins are separated from the fresh spinach leaves, soaked in cold water and washed. Spinach leaves (1000 g) were combined with 1250 ml of water in a blender, filtered through four layers of monodor polyester mesh (20 μm), and the filtrate was obtained 10 times with distilled water and adjusted the pH to 4.7 with hydrochloric acid (HCl). The electron density of the thylakoids (pH=4.7) allows for high permeability. After standing in the cold (-4 °C) for 4 hours, the thylakoids turn green and clear, yellowish yellow. The supernatant was discarded, and the precipitate containing thylakoids was collected. Subsequently, the final sediment was collected following repeated washing with centrifuges. The washed thylakoids were collected, the pH was adjusted to 7, and the final precipitates were dried to obtain a green thylakoid powder.

#### Placebo

Placebo contains corn starch, which is edible green, and like thylakoid powder, flavored with kiwi essential oil. Corn starch is a white powder that is tasteless, harmless, non-toxic, non-stimulating, and odorless, and it does not cause allergies. It is used in the food and pharmaceutical industries as an inactive (inactive) substance without therapeutic effect. Just like the thylakoids extracted from spinach, cornstarch is transformed into a green powder with a kiwi smell. Thus, thylakoid green powder or kiwi-flavored placebo are made with identical appearance (shape, size, and color). Then, they are packed in identical bags with each bag containing 5 grams of thylakoids or 5 grams of corn starch powder. Packages are coded and distributed to participants. During consumption, the contents of the bags are dissolved in a glass of water and consumed 30 minutes before lunch. Also, food notes will be taken from all subjects during the training.

#### Measurements

*Anthropometric assessment:* The participants were scheduled to attend the laboratory at the designated time to undergo an anthropometric indicator assessment. Their weight was measured

twice: first, 48 hours before the commencement of the program, and again 48 hours following the conclusion of the course. The participants were required to be barefoot during the measurement, and the scale utilized had an accuracy of 155 grams. The height of the participants was measured with a measuring tape while they stood next to a wall and without shoes, with their shoulders in a normal position, to an accuracy of 1 cm. BMI was calculated using the weight formula in kilograms per square of height in meters.

**Blood biomarkers:** Two stages of fasting blood samples were collected: pre-test (48 hours prior to the commencement of the study) and post-test (48 hours following the conclusion of the research protocol). A total of 7 ml of blood was collected from the brachial vein and transferred to tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant. Subsequently, the plasma samples underwent centrifugation (10 minutes, 3000 rpm) to separate them and transfer them to the freezer for the measurement of the target biomarkers. In this study, the levels of IL-10 (Cat. No. D194572200R, S, Biovendor, Czech Republic) and semaphorin-3E (Cat. No. SEL920Hu, Cloud-Clone Corp, USA) were quantified by ELISA (Enzyme-Linked Immunosorbent Assay) methodology (Diagnostic Biochem, Canada).

**Confounders:** At the baseline visit, only the following potential confounders were measured: age, sex, calorie intake, percentage of daily energy intake from saturated fat, and physical activity level (hours/week) at work and during leisure time.

### Data analysis

In the present study, the normal distribution of the data was shown by the Golmogorov-Smirnov test. To ascertain whether there were any statistically significant differences between the pre-test and post-test scores for each group, a paired t-test was employed. A comparison of the changes observed among the different groups was conducted using the analysis of covariance (ANCOVA) and the Bonferroni post hoc test. The data were presented as mean  $\pm$  standard deviation. All analyses were conducted using SPSS version

22 software. A p-value of less than 0.05 was considered to be statistically significant.

### Ethical considerations

The proposal for this research was approved by the Academic Center for Education, Culture and Research Organization of Khorasan Razavi. (code of ethics IR.ACECR.JDM.REC.1402.016). This study was approved by a committee of the Tehran University of Medical Sciences and its IRCT registration number is: IRCT20240608062039N1.

### Results

The present study sought to ascertain whether there were any differences in the desired variables, including body weight, BMI, and levels of IL-10 and Semaphorin-3E, between two stages of pre-test and post-test. The results of this comparison are presented in **Table 2**.

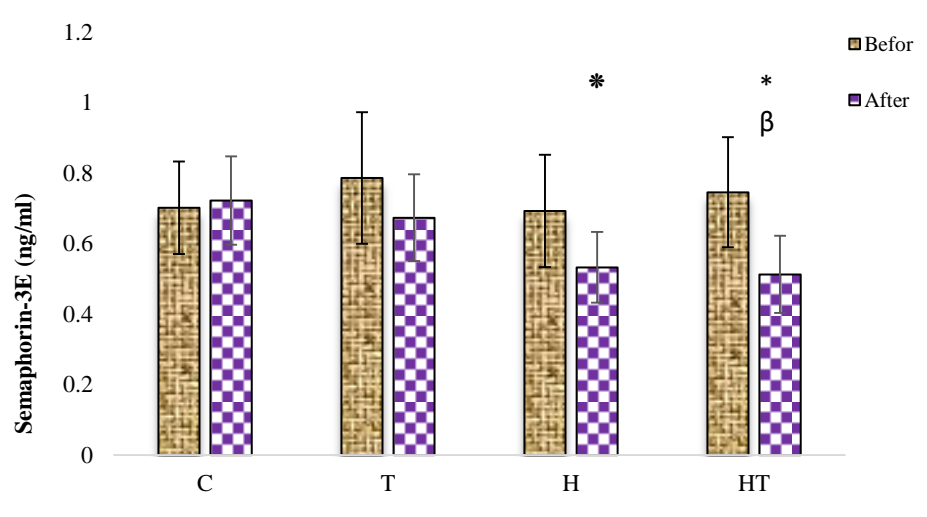
The results of the ANOVA analysis indicated a statistically significant difference between the experimental groups ( $P < 0.001$ ). Consequently, the groups were subjected to a post hoc test of Bonferroni. As illustrated in **Figure 2**, the level of Sema3E exhibited a statistically significant decline in the blood of the H and HT groups who underwent exercise in comparison to the control group ( $P < 0.01$ ). Additionally, the reduction in the level of Semaphorin-3E in the HT group that received both the supplement and the exercise program was statistically significant in comparison to the T group that only received the supplement ( $P = 0.038$ ). Nevertheless, there was no significant difference between the HT and H groups ( $P = 1$ ).

In the present study, the level of IL-10 was also evaluated as an anti-inflammatory cytokine in addition to pro-inflammatory cytokines. As illustrated in **Figure 1**, IL-10 levels exhibited a notable increase ( $P < 0.001$ ) in all three groups that received exercise, supplements, or both (groups T, H, and HT) in comparison to the control group. Furthermore, a significant difference was observed between the exercise training groups, H and HT, and the supplement group alone, i.e., T group ( $P < 0.001$ ). Furthermore, a comparison between the supplement and exercise groups (HT and H, respectively) revealed a significant difference ( $P < 0.001$ ).

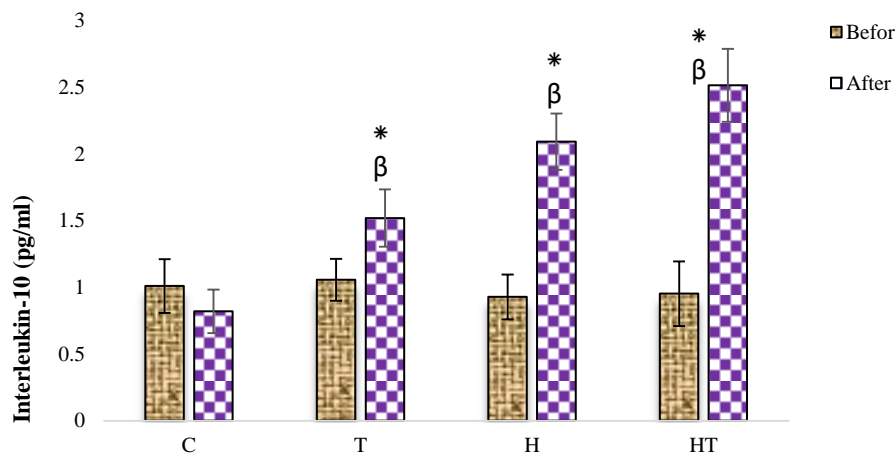
**Table 2.** Within and between comparison of studied variables in groups.

Variable/Groups	Before	After	P-value <sup>a</sup>
<b>Semaphorin-3E (ng/ml)</b>			
Control	0.701±0.039 <sup>c</sup>	0.723±0.037	0.76
Thylakoid supplement	0.786±0.056	0.674±0.037	0.03
HIFT	0.693±0.048	0.533±0.030	0.001
HIFT plus supplement	0.746±0.047	0.513±0.033	0.005
P-value <sup>b</sup>	0.001	0.001	
<b>Interleukin-10 (pg/ml)</b>			
Control	1.010±0.203	0.820±0.163	0.01
Thylakoid supplement	1.057±0.158	1.520±0.214	0.001
HIFT	0.929±0.169	2.093±0.212	<0.001
HIFT plus supplement	0.953±0.243	2.517±0.275	<0.001
P-value	0.001	0.001	
<b>Body mass index (kg/m<sup>2</sup>)</b>			
Control	33.08±1.34	32.87±1.44	0.44
Thylakoid supplement	32.66±1.37	31.93±0.93	0.01
HIFT	33.22±1.07	31.85±1.19	0.001
HIFT plus supplement	33.05±0.75	30.68±0.98	<0.001
P-value	0.001	0.001	
<b>Body weight (kg)</b>			
Control	94.33±1.82	93.55±2.43	0.37
Thylakoid supplement	93.28±2.61	91.13±2.12	0.01
HIFT	92.78±1.89	89.19±2.37	0.001
HIFT plus supplement	94.13±1.90	87.25±2.30	<0.001
P-value	<0.001	<0.001	

**HIFT:** High-intensity functional training; <sup>a</sup>: Paied t-test ; <sup>b</sup>: ANOVA test ; <sup>c</sup>: Mean ± SD.



**Figure 1.** Changes in the level of semaphorin-3E in the control group (C) without performing the exercise protocol and not receiving the supplement, the high-intensity functional training group HIFT (H group), the thylakoid supplement group (T) and the group that received the supplement in addition to exercise. \* compared to the control group and β compared to the T group. Data are shown as mean ± standard deviation.



**Figure 2.** Changes in the serum levels of interleukin 10 in the control group (C) without performing the exercise protocol and not receiving the supplement, the high-intensity functional training HIFT group (H group), the thylakoid supplement group (T) and the group that received the supplement in addition to exercise. \*compared to the control group and  $\beta$  compared to the H and T groups. Data were shown as mean  $\pm$  standard deviation.

## Discussion

The growing popularity of HIFTs has sparked interest among athletes and trainers regarding their physiological effects. This study explored the impact of combining HIFT with thylakoid supplementation on IL-10 and semaphorin-3E levels in individuals with obesity.

The study findings demonstrated that the synergy of exercise and supplementation can reduce inflammatory factors. Notably, supplementation alone effectively increased the anti-inflammatory IL-10 levels across all three exercise groups. Participants receiving both the supplement and following the exercise regimen exhibited a significant rise in IL-10 levels compared to the control group, highlighting the independent anti-inflammatory benefits of exercise and supplementation. Furthermore, a distinct difference was observed in the group combining exercise and supplementation compared to those engaging in only one intervention, emphasizing the collaborative impact of exercise and supplementation on IL-10 levels.

Previous studies by Santiago *et al.* showed a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines (Santiago *et al.*, 2021). Santiago *et al.*, in their review, noted an elevation in IL-10 levels associated with physical exercise, indicating a decrease in pro-

inflammatory cytokines related to atherosclerosis (Santiago *et al.*, 2021). Kulshrestha *et al.* emphasized the link between circulating IL-10 levels and metabolic risk factors, highlighting IL-10 as a biomarker for assessing metabolic risks (Kulshrestha *et al.*, 2018). They also identified a negative correlation between circulating IL-10 levels and BMI, suggesting a higher risk of reduced IL-10 levels in individuals with excess visceral fat mass (Kulshrestha *et al.*, 2018).

In the current study, the levels of Semaphorin-3E decreased with the influence of HIFT and thylakoid supplementation. Semaphorin-3E plays a crucial role in adipose tissue inflammation and insulin resistance, particularly in metabolic syndromes. The reduction in Semaphorin-3E levels may contribute to the decrease in C-reactive protein (CRP) levels, as CRP is synthesized in liver tissue. The anti-inflammatory properties of galactolipids in thylakoids, along with their antioxidant effects, likely account for the reduction in inflammatory markers seen in the supplemented groups (Saeidi *et al.*, 2023). Therefore, since the site of CRP synthesis is the liver tissue, the reduction of CRP in the intervention groups may be due to the reduction of Semaphorin-3E levels. In addition, several studies have shown that galactolipids in thylakoid naturally have anti-inflammatory properties, which may explain the

downregulation of inflammatory markers in groups receiving supplements (Ishii *et al.*, 2017, Pourteymour Fard Tabrizi and Abbasalizad Farhangi, 2021). The antioxidant effects of thylakoids have also been shown. These compounds can reduce oxidative stress due to their high content of molecules such as phenolic compounds, tannins, pigments (such as carotenoids and chlorophyll), and antioxidant enzymes such as superoxide desmutase (Roberts and Moreau, 2016, St-Pierre *et al.*, 2019). Therefore, it may reduce cell damage through antioxidant effects and subsequently reduce the level of inflammatory cytokines.

As a result, the obesity treatment was as short as twelve weeks, and most of the participants' anthropometric and biochemical measurements improved. Therefore, nutrition and exercise training are the first methods that should be considered in the treatment of obesity. First degree obese individuals responded better to treatment than second degree obese individuals. Moreover, the authors were able to establish a relationship with Sema3E on the molecular mechanism of obesity (Yang *et al.*, 2023).

The findings suggested that both HIFT and thylakoid supplementation offer beneficial effects on body weight management and inflammation control in obese individuals, aligning with prior studies emphasizing the positive outcomes of exercise training and dietary interventions on inflammatory markers. Future research should delve into the long-term sustainability of these interventions and their implications for effectively managing obesity-related complications (Sezgin *et al.*, 2022).

This evidence collectively highlights the intricate interplay between inflammation, weight loss interventions, and the potential protective effects on renal health in the context of diabetic nephropathy and obesity (Gong *et al.*, 2024). Future studies could investigate the optimal dosage of thylakoids for maximal anti-inflammatory benefits or explore different exercise modalities alongside dietary supplements for enhanced outcomes among diverse populations facing obesity-related challenges. This study's limitations

included the lack of control over the participants' side activities and exercises and the lack of control over individual differences and hereditary factors.

## Conclusion

This study provides valuable insights into the effects of HIFT combined with thylakoid supplementation on inflammatory markers in obese individuals. The findings indicated that this integrated approach significantly influences the levels of IL-10 and semaphorin-3E, suggesting potential mechanisms for improved metabolic health and reduced inflammation. The positive modulation of these markers highlights the therapeutic potential of HIFT and thylakoid supplementation as a viable strategy for managing obesity-related inflammation. Future research should further explore the long-term effects and underlying mechanisms, paving the way for innovative interventions aimed at enhancing health outcomes in obese populations.

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## Authors' contributions

All authors have taken responsibility for the complete content of this manuscript and have agreed to be accountable for all facets of the work, ensuring that any questions regarding the accuracy or integrity of any portion are properly investigated and addressed. They have also approved the version to be published.

## Conflict of interest

The authors declared no conflict of interests.

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## References

- Al-Mhanna SB, et al.** 2023. Effects of combined aerobic exercise and diet on cardiometabolic health in patients with obesity and type 2 diabetes: a systematic review and meta-analysis. *BMC Sports Science, Medicine and Rehabilitation*. **15** (1): 165.

- Atakan M, Kosar S, Guzel Y, Tin H & Yan X** 2021. The role of exercise, diet, and cytokines in preventing obesity and improving adipose tissue. *Nutrients*. **13** (5): 1459.
- Battineni G, et al.** 2021. Impact of obesity-induced inflammation on cardiovascular diseases (CVD). *International journal of molecular sciences*. **22** (9): 4798.
- Boachie MK, Thsehla E, Immurana M, Kohli-Lynch C & Hofman KJ** 2022. Estimating the healthcare cost of overweight and obesity in South Africa. *Global health action*. **15** (1): 2045092.
- Feito Y, Heinrich KM, Butcher SJ & Poston WSC** 2018. High-intensity functional training (HIFT): definition and research implications for improved fitness. *Sports*. **6** (3): 76.
- Feito Y, Patel P, Sal Redondo A & Heinrich KM** 2019. Effects of eight weeks of high intensity functional training on glucose control and body composition among overweight and obese adults. *Sports*. **7** (2): 51.
- Gong X, Zeng X & Fu P** 2024. The impact of weight loss on renal function in individuals with obesity and type 2 diabetes: a comprehensive review. *Frontiers in endocrinology*. **15**: 1320627.
- Henein MY, Vancheri S, Longo G & Vancheri F** 2022. The role of inflammation in cardiovascular disease. *International journal of molecular sciences*. **23** (21): 12906.
- Ishii M, Nakahara T, Araho D, Murakami J & Nishimura M** 2017. Glycolipids from spinach suppress LPS-induced vascular inflammation through eNOS and NK- $\kappa$ B signaling. *Biomedicine & Pharmacotherapy*. **91**: 111-120.
- Islam H, Neudorf H, Mui AL & Little JP** 2021. Interpreting 'anti-inflammatory' cytokine responses to exercise: focus on interleukin-10. *Journal of physiology*. **599** (23): 5163-5177.
- Kawai T, Autieri MV & Scalia R** 2021. Adipose tissue inflammation and metabolic dysfunction in obesity. *American journal of physiology-cell physiology*. **320** (3): C375-C391.
- Kulshrestha H, et al.** 2018. Interleukin-10 as a novel biomarker of metabolic risk factors. *Diabetes & metabolic syndrome: Clinical research & reviews*. **12** (4): 543-547.
- Nigam M, et al.** 2023. Evaluation of the association of chronic inflammation and cancer: Insights and implications. *Biomedicine & Pharmacotherapy*. **164**: 115015.
- Popoviciu M-S, Păduraru L, Yahya G, Metwally K & Cavalu S** 2023. Emerging role of GLP-1 agonists in obesity: a comprehensive review of randomised controlled trials. *International journal of molecular sciences*. **24** (13): 10449.
- Pourteymour Fard Tabrizi F & Abbasalizad Farhangi M** 2021. A systematic review of the potential effects of thylakoids in the management of obesity and its related issues. *Food reviews international*. **37** (5): 469-490.
- Pourteymour Fard Tabrizi F, Abbasalizad Farhangi M, Vaezi M & Hemmati S** 2021. Changes of body composition and circulating neopterin, omentin-1, and chemerin in response to thylakoid-rich spinach extract with a hypocaloric diet in obese women with polycystic ovary syndrome: a randomized controlled trial. *Phytotherapy research*. **35** (5): 2594-2606.
- Roberts JL & Moreau R** 2016. Functional properties of spinach (*Spinacia oleracea* L.) phytochemicals and bioactives. *Food & function*. **7** (8): 3337-3353.
- Saedi A, et al.** 2023. Supplementation with spinach-derived thylakoid augments the benefits of high intensity training on adipokines, insulin resistance and lipid profiles in males with obesity. *Frontiers in endocrinology*. **14**: 1141796.
- Santiago DDC, Lopes JSS, Neto AMdM & Andrade CMB** 2021. Analysis of biomarkers in response to high intensity functional training (HIFT) and high intensity interval training (HIIT): A systematic review study. *Archives of current research international*. **21** (3): 59-72.
- Sezgin G, Kar F & Uslu S** 2022. The effect of nutrition and exercise training on irisin and semaphorin-3E levels in obese patients. *Archives of physiology and biochemistry*. **128** (2): 558-567.
- St-Pierre A, et al.** 2019. Study of antioxidant

properties of thylakoids and application in UV protection and repair of UV-induced damage. *Journal of cosmetic dermatology*. **18 (6)**: 1980-1991.

**Steen EH, et al.** 2020. The role of the anti-inflammatory cytokine interleukin-10 in tissue fibrosis. *Advances in wound care*. **9 (4)**: 184-198.

**Tiwari A & Balasundaram P** 2021. Public health considerations regarding obesity In: StatPearls. StatPearls Publishing, Treasure Island (FL).

**Yabe H, et al.** 2021. Predicting a target exercise heart rate that reflects the anaerobic threshold in nonbeta-blocked hemodialysis patients: The

Karvonen and heart rate reserve formulas. *Therapeutic apheresis and dialysis*. **25 (6)**: 884-889.

**Yang Q, Wang F, Pan L & Ye T** 2023. Efficacy of traditional Chinese exercises in improving anthropometric and biochemical indicators in overweight and obese subjects: A systematic review and meta-analysis. *Medicine*. **102 (12)**: e33051.

**Zatterale F, et al.** 2020. Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. *Frontiers in physiology*. **10**: 505887.