Exercise and supplementation with anti-obesity herbal extract on IL-6 and FNCD5 genes in the soleus muscle of obese female rats

Ejercicio y suplementación con extracto de hierbas antiobesidad sobre los genes IL-6 y FNCD5 en músculo sóleo de ratas obesas

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How to cite in APA

Jalali, M., Zargani, M., Rahmati, S., Hajiaghaee, R., Helalizadeh, M., Roche, E., Celorrio San Miguel, A. M., Fernández Lázaro, D., & Azarbayjani, M. A. (2025). Exercise and supplementation with anti-obesity herbal extract on IL-6 and FNCD5 genes in the soleus muscle of obese female rats. *Retos*, *65*, 861–874. https://doi.org/10.47197/retos.v65.109550

Abstract

Introduction and Objective: Exercise and anti-obesity herbs may be effective interventions to reduce high-fat diet (HFD)-induced obesity. Our study examined the effects of exercise (aerobic/AE and resistance/RE) on FNDC5 and IL-6 expression in HFD-obese female rats supplemented with anti-obesity herbal extract (AOHME).
Methodology: Forty-two young Wistar female rats (aged 12 weeks) were randomly distributed into seven groups: (1) Control rats with a standard diet (Con-ND), (2) Control rats with HFD (Con-HFD), (3) AE-HFD, (4) RE-HFD, (5) AOHME-HFD, (6) AE-AOHME-HFD, and (7) RE-AOHME-HFD. Rats were fed with HFD for four weeks (except Con-ND), followed by four weeks of HFD (groups 2-7). AE consisted of treadmill running and RE of stair climbing. Exercises were performed 5 times/week. Supplementation (100 mg AOHME/kg, 1h post-exercise) administered in gavage and distilled water as placebo only to HFD. Rats were euthanized (48h after the last intervention) and soleus muscle removed.
Results: HFD administration induced significant body weight increase compared to Con-ND group. Weight increase was noticed as well in HFD group compared to AE-HFD, RE-HFD, AOHME-HFD, AE-AOHME-HFD, and RE-AOHME-HFD. FNDC5 and IL-6 genes presented significant higher expression in Con-ND and HFD groups compared to Con-HFD.
Conclusions: FNDC5 and IL-6 gene expression were repressed by HFD and restored by AE and RE alone or in combination with AOHME.

Keywords

Aerobic exercise; fibronectin type iii domain containing; interleukin-6; obesity; resistance exercise.

Resumen

Introducción y Objetivo: El ejercicio y las hierbas antiobesidad pueden ser intervenciones eficaces para reducir la obesidad inducida por una dieta rica en grasas (HFD). Nuestro estudio examinó los efectos del ejercicio aeróbico (AE) y el ejercicio de fuerza (RE) en la expresión de FNDC5 e IL-6 en ratas hembra alimentadas con una HFD junto con un extracto de hierbas antiobesidad (AOHME).
Metodología: Cuarenta y dos ratas Wistar hembras jóvenes (12 semanas de edad) se distribuyeron aleatoriamente en siete grupos: (1) control alimentadas con dieta estándar (Con-ND), (2) control alimentadas con HFD (Con-HFD), (3) AE-HFD, (4) RE-HFD, (5) AOHME-HFD, (6) AE-AOHME-HFD y (7) RE-AOHME-HFD. Después de cuatro semanas con HFD para inducir obesidad (excepto Con-ND), las ratas fueron alimentadas con HFD (grupos 2-7) durante 4 semanas adicionales. AE consistió en correr en cinta y el RE en subir escaleras. Los ejercicios se realizaron 5 veces por semana. La suplementación (100 mg/kg, 1 hora post-ejercicio) se administró por sonda nasogástrica a AOHME y agua destilada como placebo a HFD. Las ratas fueron eutanasiadas (48h tras la última intervención) y el músculo sóleo extraído.
Resultados: HFD indujo un aumento significativo del peso corporal en comparación con Con-ND, AE-HFD, RE-HFD, AOHME-HFD, AE-AOHME-HFD y RE-AOHME-HFD. Los genes FNDC5 e IL-6 presentaron una expresión significativamente mayor en Con-ND y el resto de grupos HFD que en el grupo Con-HFD.
Conclusiones: La expresión de FNDC5 e IL-6, reprimida con la dieta rica en grasas, se recuperó con AE y RE sólo o en combinación con AOHME.

Palabras clave

Dominio 5 de Fibronectina Tipo III; ejercicio aeróbico; ejercicio de fuerza; interleucina 6; obesidad.

Introduction

Overweight and obesity are becoming very prevalent worldwide pathologies in many social segments and age groups (Guilford et al., 2017; Guo et al., 2023). Sedentarism is one of the main causes of obesity and overweight, resulting in a decrease in aerobic capacity in a significant percentage of people suffering from these pathologies (Odek et al., 2020; Shirvani et al., 2019). As a result, obese people are more likely to suffer high blood pressure, cardiovascular disease and type 2 diabetes mellitus, all known as metabolic syndrome. The incidence is lower compared to normo-weight subjects (Kurdiova et al., 2014; Mancini et al., 2015). Meanwhile, based on the evidence obtained, it seems that regular physical activity along with herbal medicines is one of the methods of controlling obesity and associated pathologies. However, considering the wide variety of exercise programs and herbal medicines, their effects still need to be further investigated. Although the beneficial effects of metabolic adaptations due to aerobic exercises are well documented, moderate-intensity resistance exercises have also been presented as an alternative for obese people. However, the operating mechanisms during regular physical activity in obesity are not fully understood (Guilford et al., 2017; Guo et al., 2023; Kurdiova et al., 2014; Odek et al., 2020).

In this context, studies have shown that during physical activities, myocytes act as secretory organs, releasing messenger molecules called myokines (Guo et al., 2019). Irisin and interleukin-6 (IL-6) are two of the most studied myokines. Many of the positive effects of physical training are attributed to these myokines include regulation of altered metabolic processes, control of weight gain, anti-inflammatory effects, and improved insulin sensitivity. In the past few years, the influence of exercise programs on irisin concentration has been extensively studied. However, the effect of exercise on the expression of the fibronectin type III domain containing 5 (FNDC5) gene, a precursor of irisin, in obesity has been less investigated. On the other hand, IL-6 plays an instrumental role post-exercise in muscle hypertrophy and lipolysis (Ahn & Kim, 2020; Guo et al., 2019; Mancini et al., 2015). Nevertheless, the role of IL-6 after physical activity, especially during obesity caused by a high-fat diet, remains unclear.

In addition to exercise, diet and specific supplements also play a key role in obesity prevention or reduction. Today, the use of certain plant extracts to prevent or combat obesity has received much attention (Brown et al., 2015; Ellingsgaard et al., 2011). Vegetal extracts have several mechanisms, including increased lipolysis and fatty acid oxidation, and appetite reduction. One of these plant extracts to prevent obesity is green tea, a very popular drink worldwide, rich in catechins. Green tea's anti-obesity effect has been documented in both human and animal studies (Boström et al., 2012; Jung & Kim, 2014; Lira et al., 2010; Maak et al., 2021). Caraway is another plant extract used traditionally in popular Medicine. Recent reports indicate that caraway plays an important role in alleviating metabolic disorders associated with obesity. In this line, extracts from licorice rich in flavonoids, have been linked to anti-obesity properties by modulating fat metabolism and preadipocyte differentiation (Roca-Rivada et al., 2013; X.-Y. Yang et al., 2018). Extracts from bitter orange peel work as anti-inflammatory supplements due to the presence of flavonoid compounds, particularly synephrine and octopamine, which increase lipolysis and energy expenditure. In addition, bitter orange peel extract may improve glucose tolerance, lipid profile, and fasting blood sugar levels in obese model rodents (Baar et al., 2002; Liu et al., 2021; Shojaee-Moradie et al., 2007). On the other hand, red pepper extract is widely used in weight control diets due to the presence of antioxidants capsaicin and capsaicinoids (Keller et al., 2006; Pedersen, 2009). In this context, modulation of expression of FNDC5 and IL-6 by intake of these supplements and the combination with exercise has not been studied.

For this reason, the present research studies the effect of aerobic and resistance exercises along with the mentioned plant extracts on weight reduction in obese rats. Plant extracts will be included in a unique extract called Anti-Obesity Herbal Mixture Extract (AOHME), containing Glycyrrhiza glabra roots (licorice root), Camellia sinensis leaves (green tea), Carum carvi seeds (black caraway), Capsicum annuum fruits (red pepper), and Citrus aurantium fruit peel (orange peel). In addition, the expression of FNDC5 and IL-6 in soleus muscle in obese animals will be addressed simultaneously.

Method

Animals

This study included 42 young female Wistar rats (aged 12 weeks and weighing 180–200 g) obtained from the Pasteur Institute in Karaj, Iran. They were kept in a temperature-controlled room (22°C and 12 h of light/dark). They had ad libitum access to food and water (Histogen Laboratory of Tehran, Iran) in their cages. The study was carried out following the National Research "Guide for the Care and Use of Laboratory Animals." (2010) (Council et al., 2010). Also, compliance with the professional governmental guidelines, and under the supervision of the Institutional Animal Care and Use Committee (IACUC) at Central Tehran Branch University (Tehran, Iran) were done. In addition, every possible effort was made to ensure the animals did not suffer. Upon the rats became familiar with and adapted to the environment, the animals were randomly distributed into seven groups based on the number table strategy (Arnab, 2017) (six rats in each group): (1) Control group rats were fed with a standard/normal diet (Con-ND), (2) Control with high-fat diet (Con-HFD), (3) Aerobic exercise-HFD (AE), (4) Resistance exercise-HFD (RE), (5) AOHME-HFD (AOHME), (6) Aerobic exercise-AOHME-HFD (AE-AOHME), and (7) Resistance exercise-AOHME-HFD (RE-AOHME). Figure 1 depicts every stage of the study design.

Animal diets

This research used standard laboratory chows and a high-fat diet for experimental purposes. The standard diet consisted of wheat, wheat bran, rice polishing, and fish meal, containing around 25% proteins, 60% carbohydrates, and 15% fat in terms of caloric content. In contrast, the high-fat diet included typical food, beef fat, sugar, and condensed milk. The roughly composition was 14% proteins, 37% carbohydrates, and 49% fat regarding caloric value (Lasker et al., 2019). Furthermore, the rats in the HFD groups (Con-HFD, AE, RE, AOHME, AE-AOHME, RE- AOHME) continued with the HFD throughout the study protocol. On the other hand, rats in the Con-ND group were fed a normal diet for complete study. Rats' weights were measured at baseline, day 28, and day 56.

Aerobic exercise protocol

To acclimatize the rats in the AE group before starting the main exercise program, one week of running at a speed of 9 m/min and a duration of 20 min on a treadmill for rodents (Six Line, Azarakhsh Company, Iran) was considered. The main exercise program was carried out for 4 weeks, 5 sessions per week, at moderate intensity. The exercise intensity started at 55% VO2max in the first week and reached 70% VO2max in the last week. Also, the exercise duration for the entire four weeks was set at 20 min with a 0° slope. The rats' running speed reached 15 m/min in the first week and 30 m/min in the last week. After each main exercise session, 5 min of warm-up were included followed by 5 min of cooling down at a speed of 5 m/min (Table 1) (Qin et al., 2020; Vesali et al., 2021).

Table 1. The weekly protocol for aerobic exercise in rats

Conditions	Adaptation	First Week	Second Week	Third Week	Fourth Week
Warm up time (min)	5	5	5	5	5
Warm up speed (m/min)	7	7	7	7	7
Main exercise time (min)	20	20	20	20	20
Main exercise speed (m/min)	9	15	20	25	30
Grade-slope (º)	0	0	0	0	0
%VO_2max	35	55	60	65	70
Cool down time (min)	5	5	5	5	5
Cool down speed (m/min)	5	5	5	5	5

Abbreviations: m: meters; min: minutes; VO_2max: Maximum volume of oxygen; º: Grade.

Resistance training protocol

Rodent resistance exercise consisted of ladder climbing: 80-degree sloped, 110 cm height and using grid steps of 2 cm. At the top of the ladder, a chamber measuring 20 x 20 x 20 cm (length × width × height) operated as a refuge during resting periods (Peixinho-Pena et al., 2012). To induce resistance, a self-adhesive foam ribbon was encircled around the tail's proximal part and attached to a load device (lead weight). At the beginning, the rats were acclimated to the ladder through climbing exercises from the base to the peak for 5 days. For this purpose, four sets of experiments were assumed, with the rats completing 6 repetitions in each set without additional load weights. The rest duration between each set

was 2 min. As part of their first week of resistance exercises, rats used 50% of their body weight as a resistance. According to the regulation of adaptation, the amount of weight being carried was gradually increased every week until it reached 70% of the rat's body weight in the final week (which was the fourth week, as shown in Table 2). There were four sets of experiments considered, with the rats performing 8 repetitions in each set. Also, the rest period between each set was 2 min. Additionally, the rodents climbed the ladder to warm up and cool down. During their four sets, they did 10-min warm-up and 10-min cool-down repetitions without adding additional weight. The main resistance exercise program was conducted for four weeks, with five sessions a week (Gomes et al., 2017).

Table 2. The detailed specifications of the rats' weekly resistance exercise protocol.

Week	First	Second	Third	Fourth
Weights (% body weight)	50%	60%	65%	70%
Sets	4	4	4	4
Rest time between sets (min)	2	2	2	2
Number of repetitions in each set	8	8	8	8

Abbreviations: min: minutes; %: Percentage.

Plant material and extraction procedure

Glycyrrhiza glabra roots, Camellia sinensis leaves, Carum carvi seeds, Capsicum annuum fruits, and Citrus aurantium fruit peel were purchased from an authentic store. After that, the scientific names were approved by the Medical Plants Research Institute Herbarium. After milling the herbs (50 g each) in a blender, 90% ethanol was added, and a percolator was used to extract them. This process was repeated three times, and the extracts were concentrated by vacuum distillation. After calculating the amount of dried extract, it was stored in a sealed plate in the refrigerator at 4°C until the experiment. Using 90% ethanol, the concentrates of Glycyrrhiza glabra roots, Camellia sinensis leaves, Carum carvi seeds, Capsicum annuum fruits, and Citrus aurantium fruit peel concentrates were redissolved and mixed in equal proportions. This mixture was then evaporated to create a mixture of herbal extracts (MHE). A sealed container of the sample was kept in the refrigerator at 4°C until the experiment began.

Determination of the extract's total phenolic content

The total phenolic content (TPC) was determined by Folin-Ciocalteu colorimetry (Gutfinger, 1981). A plant extract solution (1 mL) was diluted with a volumetric flask with 5 mL of distilled water, followed by mixing it with 500 mL of Folin-Ciocalteu reagent. After that, one mL of 15% sodium carbonate solution was added to the mixture and let stand for 30 min. The 725 nm absorbance (Human, USA) was determined by employing an absorbance spectrophotometer. The calibration curve was generated using Gallic acid as a standard. The TPC content was expressed as mg of Gallic acid equivalents per g of extract. A triplicate of each sample was analyzed.

Yield of extraction and Polyphenol content of the herbal extracts

Using Folin-Ciocalteu's colorimetric procedure, the total phenolic content of both the herbal extracts and MHE was quantified, as outlined in Table 3.

Table 3. The yield of extraction and total phenolic content of the herbal extracts and MHE

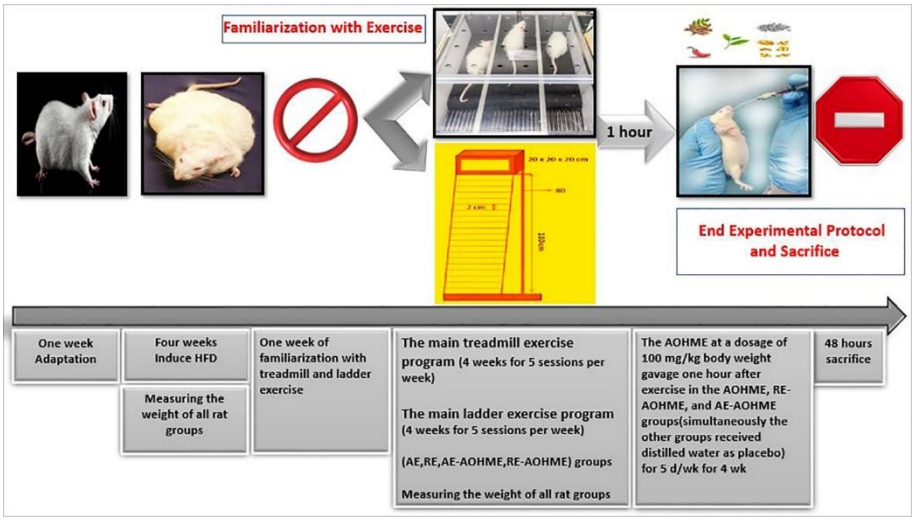
Extract	Yield of extraction (%)	Phenolic compounds (mg GA eq/g extract)
Glycyrrhiza glabra roots	15	80.34±1.67
Camellia sinensis leaf	18	151.84±2.04
Carum carvi seeds	12	75.24±1.05
Capsicum annuum fruits	11	83.76±2.03
Citrus aurantium fruit peel	10	71.69±1.16
MHE	-	98.80±2.03

Abbreviations: mg GA eq: milligrams of Galic acid equivalents; MHE: mixture of herbal extracts.
Values expressed as means ± standard deviation (SD) of triplicate measurements.

Administration of Anti-Obesity Herbal Mixture Extract (AOHME)

One hour after exercise, rats were provided with AOHME dissolved in distilled water at a dosage of 100 mg/kg body weight (Haidari et al., 2013; Hansen et al., 2011; Mir et al., 2022; Ogunruku et al., 2019; Ojha et al. 2013) by gavage in the AOHME, RE-AOHME, and AE-AOHME groups (simultaneously the other groups received distilled water as placebo) for 5 days/week during four weeks. Figure 1 indicates the experimental design.

Figure 1. Experimental design



Abbreviations: AE: Aerobic exercise group; AE-AOHME: Aerobic exercise-Anti-obesity herbal medicine extract group; AOHME: Anti-obesity herbal medicine extract group; cm: centimeter; Con-HFD: Control with high-fat diet group; Con-ND: Control with standard diet; d: day; HFD: Hight-fat diet; kg: kilogram; mg: milligram; RE: Resistance exercise group; RE-AOHME: Resistance exercise-Anti-obesity herbal medicine extract group; w: week; (n = 6 for each group).
Source: own creation.

Tissue sampling

Forty-eight hours after the last intervention, and after 8-10 h of fasting, the rats were weighed. Then they were anesthetized with ketamine (50 mg/kg body weight) and xylazine (5 mg/kg body weight). Upon total anesthesia, the rats were euthanized and the soleus muscle tissue was removed and transferred to liquid nitrogen. Finally, it was kept at -85ºC until determinations were conducted.

RNA extraction and quantitative real-time PCR

One hundred mg of soleus muscle tissue was extracted with Qiazol (Qiazol lysis reagent, USA) in a sterilized RNase-free tube. The RNA concentration and purity were measured with a NanoDrop ND-100 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Using a Revert Aid cDNA synthesis kit from Fermentas, Germany, the RNA was transformed into cDNA in a 25-mL volume following the manufacturer's instructions. We performed polymerase chain reactions (PCRs) with 2 mL of cDNA synthesis, 12.5 mL of AccuPrime SuperMix I (Fermentas, Germany), and 0.2 mL of forward primer and reverse primer (both at 100 mol/L). Primer3 software and NCBI BLAST Instrument were used to design and confirm the primers. An analysis of relative gene expression was conducted by real-time PCR with 500 ng of freshly synthesized cDNA. SYBR Green Premix 2 (Takara, Shiga, Japan) and 10 pM mixed primers (25 L) were used for the PCR reaction. The thermocycling process involved denaturation at 95°C for 10 seconds, 94°C for 5 seconds, and annealing and extension at 60°C for 34 seconds. To determine the relative expression of the IL-6 and FNDC5 genes, the CT of the samples was compared to the CT of the internal control, GAPDH (glyceraldehyde-3-phosphate dehydrogenase). PCR was performed with an ABI detection system (Applied Biosystems, USA). Five replicates were performed for each reaction. We double-checked the PCR reaction specificity using electrophoresis and melting curve analysis. Table 4 lists the primer pairs used to analyze cDNA.

Table 4. Primer sequences used for real-time PCR

Gene	Forward	Reverse	Amplicon Size, bp
FNDC5	CTCTCCTGGCTTTCCTCTTTC	ATTCTGCAACTCTGTCTCTGAG	119, 2179
IL-6	ACCAAGACCATCCAACTCATC	GCTTAGGCATAGCACACTAGG	94, 1045
GAPDH	AAGTTCAACGGCACAGTCAAGG	CATACTCAGCACCAGCATCACC	

Abbreviations: bp: base pair; FNDC5: Fibronectin type III domain containing 5; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; IL-6: Interleukin-6.

Data analysis

Data are expressed as mean ± standard deviation (SD). Before data analysis, the normality of data distribution and homogeneity of variances were analyzed using the Shapiro-Wilk and Levene tests, respectively. Statistically, to evaluate the effect of HFD on studied genes, the Con-ND group was compared with the Con-HFD group using an independent t-test. A significant difference test for weight was performed with a two-way analysis of variance (ANOVA) with repeated measures. In case of a significant interaction between groups and time, Bonferroni's post-hoc test was applied. One-way analysis of variance was used to test the significant difference in FNDC5 and IL-6 gene expression between groups. The Bonferroni's post hoc test was used for pairwise comparisons. A significance level of $p<0.05$ was considered for all calculations. The calculations were performed using SPSS 25 software.

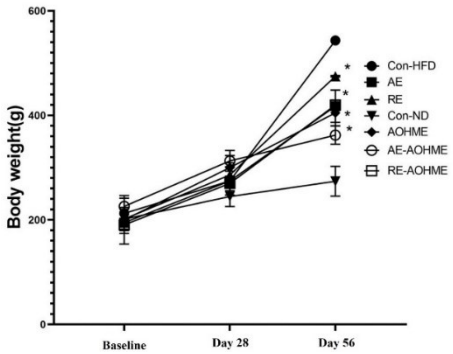
Sample Size

Post hoc power calculations were used instead of a priori sample size estimation in this study by G Power3 power analysis program, Düsseldorf, Germany. Post-hoc power analysis revealed that the statistical power for the effect of exercise, AOHME, and exercise*AOHME on FNDC-5 and IL-6 was between 0.7 and 0.9. Therefore, sample size did not negatively affect statistical power.

Results

Shapiro-Wilk and Levene tests showed that the data distribution was normal, and the variances were homogeneous. HFD feeding caused a significant increase in animals' weight at the end of the eighth-week period in comparison with Con-ND (543.48±6.70 vs 273.98± 28.65, [49.58% increase] p=0.001). The weight in AE (417.49±30.84 vs 543.48±6.70, p=0.001), RE (474.86±10.19 vs 543.48±6.70, p=0.017), AOHME (404.71±24.74 vs 543.48±6.70, p=0.001), AE-AOHME (362.25±17.44 vs 543.48±6.70, p=0.001), and RE-AOHME (419.85±11.73 vs 543.48±6.70, p=0.001) groups was significantly lower than in the Con-HFD group at the end of the experimental period. From higher to lower weight loss was observed in the AE-AOHME group (33.34% decrease), AOHME group (25.53% decrease), AE group (23.18% decrease), RE-AOHME group (22.74% decrease), and RE group (12.62% decrease), respectively (Figure 2). Therefore, in rats fed with HFD for 28 and 56 days, AE, RE, and AOHME supplementation affected body weight.

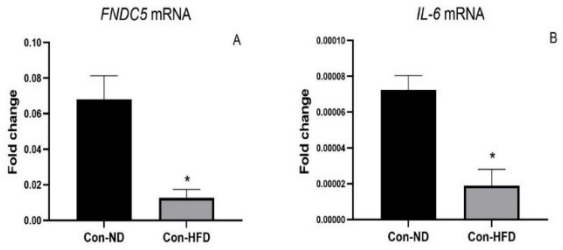
Figure 2. Weight evolution of Wistar rats throughout the study



Abbreviations: AE: Aerobic exercise group; AE-AOHME: Aerobic exercise-Anti-obesity herbal medicine extract group; AOHME: Anti-obesity herbal medicine extract group; Con-HFD: Control with high-fat diet group; Con-ND: Control with standard diet; RE: Resistance exercise group; RE-AOHME: Resistance exercise-Anti-obesity herbal medicine extract group; g: grams; (n = 6 for each group).
Data are expressed as mean ± standard deviation (SD); Two-way analysis of variance (ANOVA) with repeated measures, post hoc Bonferroni's test; *Significantly different with respect to the Con-HFD group ($p < 0.05$)
Source: own creation.

HFD feeding caused a significant decrease in FNDC5 gene expression (0.012 ± 0.004 vs 0.068 ± 0.013, p=0.001) and IL-6 gene expression (0.000018 ± 0.000009 vs 0.000072 ± 0.000008, p=0.001) compared to the Con-ND group (Figure 3A and 3B).

Figure 3. Fibronectin type III domain containing 5 (FNDC5) [Pannel A] and Interleukin-6 (IL-6) [Pannel B] mRNA expression in the soleus muscle of rats fed with normal (Con-ND) and high-fat (Con-HFD) diets.

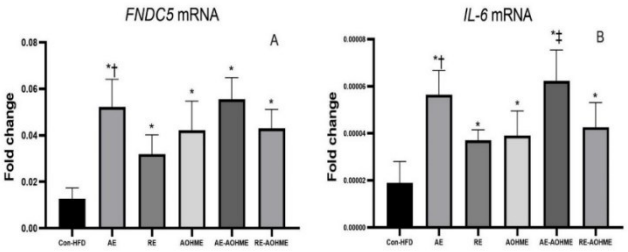


Abbreviations: Con-HFD: Control with high-fat diet group, Con-ND: Control with standard diet; FNDC5: Fibronectin type III domain containing 5; IL-6: Interleukin-6; (n = 6 for each group).
Data are expressed as mean ± standard deviation (SD); Independent t test; *Significantly different with respect to the Con-ND group ($p < 0.05$).
Source: own creation.

FNDC5 gene expression in the AE (0.052 ± 0.011 vs 0.012 ± 0.004, p=0.001) and RE (0.031 ± 0.008 vs 0.012 ± 0.004, p=0.034) groups was significantly higher than in the Con-HFD group. Additionally, the AE group presented significantly higher expression of FNDC5 gene than the RE group (0.052 ± 0.011 vs 0.031 ± 0.008, p=0.02). Consumption of AOHME caused a significant increase in FNDC5 gene expression compared to the Con-HFD group (0.042 ± 0.012 vs 0.012 ± 0.004, p=0.001). FNDC5 gene expression in the AE-AOHME group (0.055 ± 0.009 vs 0.012 ± 0.004, p=0.001) and RE-AOHME group (0.043 ± 0.008 vs 0.012 ± 0.004, P=0.001) was significantly higher than in the Con-HFD group. FNDC5 gene expression was not significantly different between the AE-AOHME and RE-AOHME groups (0.055 ± 0.009 vs 0.043 ± 0.008, p=0.350) (Figure 4A).

In addition, a significant increase in IL-6 gene expression was observed in the AE group (0.000056 ± 0.00001 vs 0.000018 ± 0.000009, p=0.001) and RE group (0.000037 ± 0.000004 vs 0.000018 ± 0.000009, p=0.043) compared to the Con-HFD group. IL-6 gene expression in the AE group was significantly higher than in the RE group (0.000056 ± 0.00001 vs 0.000037 ± 0.000004, p=0.023). AOHME consumption significantly increased IL-6 gene expression compared to the Con-HFD group (0.000038 ± 0.00001 vs 0.000018 ± 0.000009, p=0.019). The combination of AE-AOHME (0.000062 ± 0.000013 vs 0.000018 ± 0.000009, p=0.001) and RE-AOHME (0.000042 ± 0.00001 vs 0.000018 ± 0.000009, p=0.003) increased IL-6 gene expression compared to the Con-HFD group. IL-6 gene expression in the AE-AOHME group was considerably higher than in the RE-AOHME group (0.000062 ± 0.000013 vs 0.000042 ± 0.00001, p=0.022) (Figure 4B).

Figure 4. Fibronectin type III domain containing 5 (FNDC5) [Pannel A] and Interleukin-6 (IL-6) [Pannel B] mRNA expression in the rats's soleus muscle after 4 weeks.



Abbreviations: AE: Aerobic exercise group; AE-AOHME: Aerobic exercise-Anti-obesity herbal medicine extract group; AOHME: Anti-obesity herbal medicine extract group; Con-HFD: Control with high-fat diet group; FNDC5: Fibronectin type III domain-containing protein 5; IL-6: Interleukin 6; RE: Resistance exercise group; RE-AOHME: Resistance exercise-Anti-obesity herbal medicine extract group; (n = 6 for each group).
Data are expressed as mean ± standard deviation (SD); One-way analysis of variance (ANOVA) with repeated measures, post hoc Bonferroni's test; *Significantly different ($p < 0.05$) compared to Con-HFD group; †Significantly different ($p < 0.05$) compared to RE group; ‡Significantly different ($p < 0.05$) compared to RE-AOHME group.
Fuente: own creation.

Discussion

Rats fed with HFD showed a significant decrease in expression of FNDC5 and IL-6 genes in the soleus muscle compared to control rats (Con-ND). The results of studies regarding the effect of HFD feeding and the expression of the FNDC5 gene are largely inconsistent. Some studies have shown an increase in FNDC5 expression in response to HFD (Kazeminasab et al., 2021; Kazeminasab et al., 2018; Xiong et al., 2018), contrary to our results and others' results. In this regard, the study by Guo et al. revealed that FNDC5 expression in muscle tissue or C2C12 myotubes was reduced due to HFD feeding (Guo et al., 2023). Also, the investigation conducted by de de Macêdo et al. indicated a significant decrease in FNDC5 mRNA and protein expression levels in the soleus muscle of rodents exposed to HFD feeding (de Macêdo et al., 2017; Guo et al., 2019; Lasker et al., 2019). Altogether, it seems that elevated HFD consumption, consistent with previous research, led to a reduction in FNDC5 gene expression. The expression of IL-6 gene expression in muscle tissue was also significantly reduced by HFD feeding. In this line, decreased IL-6 gene expression in muscle tissue has been reported in animals fed with HFD (Ahn & Kim, 2020; Brown et al., 2015; de Macêdo et al., 2017; Moreno-Navarrete et al., 2013; Z. Yang et al., 2015). As IL-6 plays a biological role in muscle, especially in myoblast proliferation and differentiation, this could explain the related alterations in muscle function in obese subjects. In addition, IL-6 is secreted post-exercise by the muscle as a myokine, stimulating the production of glucagon-like peptide-1 (GLP-1), controlling satiety and blood glucose levels. This action suggests a possible link between exercise and body weight control in obesity (Ellingsgaard et al., 2011). Therefore, it can be considered that the decrease in the expression of the muscle IL-6 gene under HFD is one of the key factors in the occurrence of metabolic disorders associated with HFD-induced obesity.

In addition, this research revealed that both AE and RE enhanced FNDC5 gene expression in the soleus muscle under HFD conditions. The increase in FNDC5 gene expression was higher in the AE group than in the RE group. AE develops an array of phenotypes in skeletal muscle for optimal adaptation to oxidative situations. One of the oxidative changes in skeletal muscle is the increased expression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α). PGC-1α is involved in many metabolic adaptations resulting from physical activities such as mitochondrial biogenesis, thermogenesis, and energy metabolism (Jung & Kim, 2014; Lira et al., 2010). Evidence shows that increasing PGC-1α enhances FNDC5 gene expression in skeletal muscle tissue (Maak et al., 2021). Activation of PGC-1α in response to physical activities is the main mechanism for enhancing the level and cleavage of FNDC5 from skeletal muscle tissue (Boström et al., 2012). It has been reported that both one session of running on the treadmill (acute effect) and eight weeks of running (chronic effect), increased PGC-1α and FNDC5 gene expression (Dehghani et al., 2018). Therefore, FNDC5 gene expression in muscles positively correlates with aerobic performance (Lecker et al., 2012).

On the other hand, evidence shows that although aerobic training increases in both types of fast-twitch and slow-twitch muscle fibers, the expression level of FNDC5 gene is higher in slow-twitch muscles with high aerobic capacity (Roca-Rivada et al., 2013). As indicated in the current research, FNDC5 gene expression was measured in the soleus muscle as a slow-twitch muscle. Its increased expression was consistent with previous studies (Norheim et al., 2014; Rahmati-Ahmadabad et al., 2021). Another mechanism for enhancing FNDC5 gene expression due to aerobic exercise is the cAMP response element-binding protein (CREB), which is a transcription factor that in C2C12 myotubes can control FNDC5 expression by binding to PGC-1α and increasing FNDC5 gene expression (X.-y. Yang et al., 2018). Moreover, this study showed that RE also resulted in an increase in FNDC5 gene expression. Evidence shows that, like AE, RE also increases FNDC5 gene expression in skeletal muscle by enhancing PGC1α gene expression (Liu et al., 2021). It has been reported that during muscle contraction, the increase in the influx of calcium ions (Ca2+) increases the expression and activity of PGC-1α (Baar et al., 2002). Since PGC-1α causes an increase in FNDC5 gene expression, the increase in the FNDC5 gene can be justified as a result of RE. As mentioned previously, HFD inhibits FNDC5 gene expression in muscle tissue. This effect is reverted by both types of exercise, more prominently in AE. This is explained because AE could increase fat oxidation in active muscle compared to RE, a type of exercise that relies less on fat oxidation. Therefore, in the present study, compared to the RE group, the increase in the expression of the FNDC5 gene and the decrease in the inhibitory effect of HFD in the AE group could be explained (Guo et al., 2023; Muscella et al., 2020).

Like FNDC5, both AE and RE increased IL-6 gene expression in this study under HFD conditions. Active skeletal muscle synthesizes IL-6 as a myokine and releases it into circulation post-exercise. This causes many adaptations as well as health and physical performance enhancements (Pedersen, 2009). Several mechanisms have been proposed to explain IL-6 expression in response to physical activity. One proposed mechanism to activate IL-6 gene expression could be mediated through the increase in cytosolic free calcium in working muscles (Keller et al., 2006). Another mechanism to justify the increase in IL-6 gene expression due to exercise is the increase in ATP production in muscle cells (Fernández-Verdejo et al., 2014). Therefore, activation of beta2-adrenergic receptors (ADRB2) influences IL-6 production in the skeletal muscle (Hostrup et al., 2022). It is well known that physical exercise, especially AE, is one of the main triggers of ADRB2 activation (Azevedo et al., 2021). The increase in the suppressor of cytokine signaling-3 (SOCS-3) as a result of physical exercise is another mechanism for increasing IL-6 gene expression (Spangenburg et al., 2006). As revealed in this research, rats fed HFD lost weight significantly due to exercise, especially AE. It has been found, however, that IL-6 gene expression increased in rats fed HFD along with aerobic and resistance exercise (Ahn & Kim, 2020).

In addition to exercise, herbal supplements can also increase the expression of FNDC5 and IL-6. In this line, AOHME consumption increased FNDC5 gene expression. The AOHME used in this study contained green tea, black caraway, licorice, bitter orange peel, and red pepper. According to the Introduction, these 5 extracts are effective in preventing obesity. In this line, AOHME phytochemical compounds stimulate FNDC5 gene expression through the activation of PGC-1α gene expression. Lee et al. showed that epigallocatechin-3-gallate found in green tea stimulates the expression of the PGC-1α gene in liver cells and adipocytes (Lee et al., 2016). It was also reported that capsaicin found in red pepper, through the transient receptor potential vanilloid 1 (TRPV1), stimulates the PGC-1α gene expression in skeletal muscle (Luo et al., 2012). In rats fed HFD, capsaicin increases PGC-1α gene expression (Kang et al., 2010). Bitter orange peel contains a biogenic amine called octopamine. It has been reported that in rats fed with deep frying oil, octopamine increased the expression of the myocardial PGC-1α gene (Kianmehr et al., 2020).

Regarding IL-6, AOHME increased gene expression. Since physical exercise enhances IL-6 gene expression by increasing intracellular calcium, evidence shows that capsaicin in red pepper raises intracellular calcium and stimulates IL-6 gene expression in skeletal muscle by activating TRPV1 (Obi et al., 2017). ADRB2 activation by AOHME can be considered one of the possible mechanisms for enhancing IL-6 gene expression. Evidence shows that capsaicin (Kawada et al., 1986), and p-synephrine (Stohs et al., 2011) activate ADRB2, increasing muscle IL-6 gene expression. Moreover, red pepper consumption in mice fed with HFD has led to weight loss and an improved blood lipid profile (H.-J. Kim et al., 2020). Even, studies on rodents have demonstrated that capsaicin causes an increase in fatty acid utilization and endurance swimming capacity in mice. Besides, administration of capsaicin in rats fed with HFD led to a decrease in body weight and epididymal adipose tissue (K.-M. Kim et al., 1997; Seo et al., 2003). On the other hand,

human studies showed that capsaicin supplementation improves muscular endurance, and even animal models fed with HFD could ameliorate hyperlipidemia and atherosclerosis (Grgic et al., 2022; S. Yang et al., 2019). Black caraway has been characterized as an anti-obesity supplement with beneficial effects such as reducing total cholesterol levels, triglycerides, and low-density lipoproteins (LDL) (Bansal et al., 2023; Khaksari et al., 2014). Consistent with licorice's function and according to studies performed on C57BL/6J mice, ethanolic extract of licorice led to improved metabolic syndrome, diabetes, moderate abdominal obesity, and high blood pressure (Mae et al., 2003).

Moreover, the present study indicates that it is possible to combine exercise with anti-obesity plant extracts to restore FNDC5 and IL-6 expression in the context of HFD. However, the differences compared to exercise alone are not significant. AE in combination with AOMHE results in higher myokine expression levels. The results of some studies in humans support the use of both strategies together. For instance, licorice consumption along with AE in overweight women improved antioxidant capacity, BMI, and weight loss (Asjari et al., 2021). Myokines were not studied in this report. However, according to the evidence from published studies and the results of the present study, it seems that HFD decreases myokines gene expression and results in weight gain. However, when weight loss strategies (exercise and/or anti-obesity herbal supplements) were executed myokine expression increased, especially IL-6 (Shin et al., 2015). In addition, the highest level of FNDC5 and IL-6 gene expression was observed in the groups receiving AOHME and AE-AOHME, indicating that physical activity (particularly aerobic) potentiates AOHME's anti-obesity action.

However, the present study had some limitations. Weight loss is a complex process, and additional measurements, like irisin, UCP-1, and lipid profile, can provide a more complete picture. This could help to decipher the transduction pathway operating under these conditions for weight reduction. In addition, the levels of sex hormones and catecholamines were not determined, to decipher the influence of sex on weight management. However, in the context of the effect of resistance and endurance training with AOHME in animal models fed with HFD, this study provides an innovative perspective on improving obesity through exercise along with the role of AOHME.

Conclusions

In this preliminary study, we found that HFD feeding reduces FNDC5 and IL-6 gene expression. By releasing FNDC5/irisin, together with IL-6, can restore metabolic disorders caused by HFD and energy metabolism. Performing aerobic and resistance exercises together with AOHME (separately or in combination) could restore FNDC5 and IL-6 gene expression, repressed by HFD. This combination could prevent metabolic disorders caused by obesity. A consistent effect was observed when exercise was accompanied by AOHME. Based on this, it is recommended that exercise, especially aerobic exercise with AOHME, can be a suitable solution to reduce obesity complications in skeletal muscle tissue. However, further investigation into the proposed interventions is necessary to confirm the results.

Acknowledgements

The authors would like to thank the Recognized Research Group "Neurobiology" of the Faculty of Medicine of the University of Valladolid for their support and involvement in this study. CIBEROBN is an initiative of Instituto de Salud Carlos III, Spain.

Financing

This research was funded by the Department of Education of the Junta de Castilla & León and the European Regional Development Fund (ERDF) by TCUE Plan 2023–2027 (grant no. 067/230003). The principal investigator was Prof. Dr. Diego Fernández-Lázaro.

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