چهارمین کنفرانس بین‌المللی دانشجویان
4th International Conference form
Students of Sports Sciences
and Physical Education
علوم ورزشی
و تربیت بدنی
www.sport.cdsts.ir

The Effect of 8 Weeks of High-Intensity Interval Training on the Gene Expression of Hepatokines in Male Wistar Rats with Non-Alcoholic Fatty Liver Disease

Fatemeh Bazzar [1,*] - Ali Younesian [2] - Elaheh Talebi Garkani [3]
[1] *M.Sc. Student, Faculty of Physical Education and Sports Sciences, Exercise Physiology, Shahrood University of Technology, Iran*
[2] *Associate Professor, Department of Exercise Physiology, Faculty of Physical Education and Sports Sciences, Shahrood University of Technology, Iran*
[3] Associate Professor, Department of Exercise Physiology, University of Mazandaran, Babolsar, Mazandaran, Iran

ABSTRACT

Objectives: Non-alcoholic fatty liver disease (NAFLD) is closely associated with hepatokines such as fibroblast growth factor 21 (FGF21), follistatin, and leukocyte cell-derived chemotaxin 2 (LECT2), which play roles in regulating liver metabolism. Exercise training has been proposed as a non-pharmacological approach for managing NAFLD. The aim of this study was to investigate the effects of 8 weeks of high-intensity interval training (HIIT) on the gene expression of hepatokines in a dexamethasone-induced animal model of NAFLD.

Materials and Methods: In this study, 24 male Wistar rats were randomly divided into 3 groups of 8 rats each: 1- healthy control, 2- fatty liver control, 3- fatty liver + high-intensity interval training. Except for the healthy control group, disease induction was performed in the other groups. The high-intensity interval training protocol was implemented incrementally for 8 weeks, 5 sessions per week. Hepatic gene expression of FGF21, follistatin, and LECT2 was measured using real-time PCR, and data were analyzed with one-way analysis of variance.

Results: The results showed no significant differences in the gene expression levels of follistatin and LECT2 among the study groups, although the relative expression of the FGF-21 gene was higher in the fatty liver control group compared to the healthy control, but it was not statistically significant $P \leq 0.05$.

Discussion: Despite improvements in overall health indices, HIIT did not alter the gene expression of hepatokines in the dexamethasone-induced NAFLD model. The results suggest that observing significant changes in hepatokine gene expression may require longer training periods, more standardized NAFLD models, or different training modalities.

Keywords: Follistatin, Fibroblast Growth Factor 21, High-Intensity Interval Training, Leukocyte Cell-Derived Chemotaxin 2, Non-Alcoholic Fatty Liver Disease

1. *INTRODUCTION*

Non-alcoholic fatty liver disease (NAFLD) is a reversible metabolic disorder characterized by excessive accumulation of triacylglycerols in the liver (1).The spectrum of NAFLD ranges from hepatic steatosis (fatty liver) to non-alcoholic steatohepatitis (NASH) and liver cirrhosis (2). This disease primarily affects individuals with visceral obesity, dyslipidemia, insulin resistance, or impaired glucose tolerance and is considered one of the manifestations of metabolic syndrome (3). The liver is an essential organ in fat metabolism and serves as the central regulator of lipid homeostasis, responsible for the synthesis of new fatty acids, their transport and redistribution to other tissues, as well as their utilization as energy substrates (4). The liver also releases specific proteins called hepatokines, some of which play roles in the metabolic homeostasis of the entire body and appear to be involved in the development of metabolic diseases (5). Hepatokines can have beneficial or detrimental effects on metabolic health by regulating energy-related

signaling pathways (6,7).In other words, it has been shown that the liver influences lipid and glucose metabolism by releasing hepatokines into the blood, and NAFLD appears to be associated with alterations in hepatokine production (3). Fibroblast growth factor 21 (FGF21) is a hepatokine primarily secreted from the liver and acts on various tissues (8). FGF21 directly regulates fat metabolism and reduces hepatic fat accumulation in an insulin-independent manner (3). Additionally, FGF21 reduces potential NAFLD risk factors, including obesity, dyslipidemia, and insulin insensitivity. Therefore, FGF21 is a potential pharmaceutical candidate for diseases such as NAFLD, dyslipidemia, and type 2 diabetes (8). Leukocyte cell-derived chemotaxin 2 (LECT2) is another hepatic hepatokine whose plasma levels are higher in individuals with type 2 diabetes and NAFLD compared to healthy individuals (9,10). In fact, serum LECT2 levels reflect hepatic fat content (11). Deletion of the LECT2 gene in mice has been shown to improve insulin sensitivity (11). Follistatin is also a hepatokine expressed in the liver, serving as one of the main regulators of liver biology and playing a significant role in liver pathology and regeneration (12). It has been observed that follistatin is associated with metabolic diseases, and its plasma levels are elevated in patients with type 2 diabetes, NAFLD, and NASH (13). Physical activity and exercise can potentially be effective in managing NAFLD by reducing intrahepatic lipid (IHL) content. Cross-sectional studies have shown that higher levels of physical activity are associated with lower IHL levels (14). In this regard, it has been reported that 12 weeks of high-intensity interval training (HIIT) significantly reduces intrahepatic fat levels (15,16). Since the liver is the center of metabolism, physical activity can alter the synthesis and secretion of hepatokines and affect whole-body homeostasis (6,7). Regarding changes in hepatic hepatokines in response to exercise, based on our search, there are very few studies. In one study, changes in LECT2 were examined after a period of aerobic-resistance exercise training and it was shown that its plasma levels were significantly reduced in obese women with non-alcoholic fatty liver disease (17), whereas plasma levels remained unchanged after a single bout of intense and moderate physical activity in healthy men (18). It has also been shown that a single session of high-intensity exercise produces a greater increase in circulating FGF21 compared to moderate intensity (18), And in obese mice, 4 weeks of aerobic and resistance training significantly increased FGF21 compared to the control group (19). Furthermore, plasma follistatin also increased after physical activity, independent of exercise intensity (18). However, its hepatic expression in mice fed a high-fat diet was lower after a period of aerobic exercise compared to the sedentary control group (20). Therefore, since the results of existing studies appear contradictory and changes in hepatic hepatokines in response to exercise training have not been clearly defined, and given that exercise intensity is a key variable and protocols with higher intensity typically produce the greatest therapeutic effects (21), considering the scarcity and inconsistency of findings, and the importance of exercise intensity as a key variable, the aim of this study is to investigate the effect of 8 weeks of high-intensity interval training on the gene expression of FGF21, follistatin, and LECT2 in rats with non-alcoholic fatty liver disease. This study can help better understand the effect of high-intensity exercise on hepatokines and their interaction with metabolic disorders.

2 Materials and Methods

2.1Study Subjects

In this study, twenty-four male Wistar rats were obtained from the Pasteur Institute of Iran and housed in an environment with a temperature of 22 ± 1°C, humidity of 55%, and a 12:12 light-dark cycle (12 hours light, 12 hours dark) in standard polycarbonate cages (4 rats per cage). After one week of acclimation to the laboratory environment, the animals were divided into 3 groups of 8 rats each based on weight matching (initial average weight: 230 grams): 1- healthy control, 2- fatty liver control, 3- fatty liver + high-intensity interval training. Except for the healthy control group, induction of non-alcoholic fatty liver disease was performed in the other two groups. All care procedures were conducted in accordance with the guidelines for the use and care of laboratory animals, and ethical principles were observed. Additionally, this project was reviewed by the Research Ethics Committee of the University of Mazandaran and approved with the code IR.UMZ.REC.1399.018.

2.2 Induction of Fatty Liver

To induce fatty liver, a synthetic glucocorticoid, dexamethasone (Darou Pakhsh Pharmaceutical Company, Iran), was injected at a dose of 10 mg per kg body weight for 4 days in the rats (22). Before implementing the protocol, dexamethasone induction was piloted on 3 rats to assess the degree of fatty liver. After examining histological samples and confirming fat droplets in the liver tissue, disease induction was performed on the diseased groups.

2.3 Training Protocol

The animals in the training groups began the high-intensity interval training (HIIT) protocol after one week of familiarization with the treadmill (three sessions per week, 10 minutes each). The high-intensity interval training consisted of 6 bouts of 3-minute running at 40 m/min with 3-minute active recovery at 20 m/min between bouts at a 15% incline. In this protocol, speed, incline, and activity duration were progressively increased. The initial speed started at 15 m/min, and 1-2 m/min was added per session based on the rats' ability. By the end of the fifth week, the speed reached 40 m/min and remained constant until the end of the training period. The treadmill speed during active recovery started at 12 m/min, with 1-2 m/min added each week. The training lasted 8 weeks, with a frequency of 5 sessions per week. The current protocol is a modified version of previous research protocols (23).

2.4 Anesthesia and Tissue Extraction Method

After 8 weeks, at the end of the training period and 48 hours after the last training session, and under fasting conditions (8-12 hours), the rats were anesthetized by intraperitoneal injection of a combination of ketamine solution (30-50 mg per kg) and xylazine (3-5 mg per kg). The liver tissue was quickly removed, washed with physiological saline, and transferred to microtubes. It was then frozen in liquid nitrogen and transferred to a freezer at -80°C.

2.5 RNA Extraction, cDNA Synthesis, and Gene Expression

Total RNA was extracted from liver tissue using the Denazist kit. To RNase & DNase-free microtubes, 100 mg of the homogenized sample was added, followed by 1 ml of lysis buffer (DR1). The samples were then incubated for 5 minutes. The microtubes containing the samples were centrifuged at 12,000 rpm for 10 minutes at 4°C. The supernatant was transferred to new microtubes. The resulting pellet, containing tissue fragments, was discarded, and 200 µl of chloroform was added per ml of DR1 buffer. The mixture was vigorously shaken for 15 seconds and incubated at room temperature for 3 minutes. The mixture was centrifuged at 12,000 rpm for 15 minutes at 4°C. After centrifugation, three phases formed. The upper aqueous phase contained RNA. This phase was carefully separated and transferred to a new microtube. To the new microtubes, ethanol was added in half the volume of the transferred liquid, mixed well, and transferred to a column placed inside a collection tube. The microtubes were then centrifuged at 8,000 rpm for 2 minutes. The liquid collected in the collection tube was discarded, and the column was returned to the collection tube. 500 µl of DR2 buffer was added to the column and centrifuged at 10,000 rpm for 1 minute. The liquid collected in the collection tube was discarded, and the column was returned to the collection tube. After adding 200 µl of DR2 buffer, the column and collection tube were centrifuged at 13,000 rpm for 3 minutes. Next, the column was placed in a new microtube. 50 µl of DR3 buffer was added to it, and after 2 minutes, it was centrifuged at 1,000 rpm for 2 minutes. The liquid collected at the bottom of the microtube was transferred back to the column, and after 2 minutes of incubation at laboratory temperature, it was centrifuged at 13,000 rpm for 2 minutes. The resulting solution contained RNA. The quality and quantity of the extracted RNA were evaluated using agarose gel electrophoresis and UV spectrophotometry (Nanodrop, Mabna Iranian, Iran). The extracted RNA was transferred to a freezer at -80°C until further experimental steps. The extracted RNA samples were then used for cDNA synthesis after removing genomic contaminants with DNase I, RNase-free enzyme (SinaClon, Iran).

In this study, cDNA synthesis was performed using the Yekta Tajhiz Azma kit (Iran) according to the company's instructions. For this purpose, 2 µg of total RNA was synthesized into cDNA using Oligo-dT primer according to the manufacturer's instructions. The tubes containing cDNA were stored at -80°C until use in real-time PCR reactions. In this research, specific primers for amplifying the FGF21, LECT2, Follistatin, and HPRT1 genes were designed using Primer 5 software, and the primer sequences were subsequently synthesized by Bioneer Company (South Korea). The forward and reverse primer sequences for the above genes are shown in Table 1.

Table 1. Primers Used in the Real-Time PCR Process

Gene name	Sequence	Access number	Product Length (Base Pairs)
Fgf21	F-5'-GTGCGAGGCATACCCCATC-3' R-5'-TTCGGTGTCCTGGTCGTCAT-3'	NM_130752.1	100
Lect2	F-5'-ACTCTACTCAAAGAACCCAAAGGC-3' R-5'-GCAGAAACCTCTTCCCGACA-3'	NM_001108405.1	167
Follistatin (FST)	F-5'-GAACCCTCATCTTCAGAGCAGTC-3' R-5'-CACTTTCCCTCATAGGCTAATCC-3'	NM_012561.3	125
HPRT1	F 5′-TTCTTTGCTGACCTGCTGGAT -3′ R 5′-TATGTCCCCCGTTGACTGGT-3′	NM_012583.2	123

Following the optimization of standard PCR conditions, including the determination of the optimal annealing temperature for the target genes, Real-Time PCR was performed using the Corbett Rotor-Gene 6000 instrument and the Sybergreen kit.

The Real-Time PCR reactions were carried out in a final volume of 20 µl, with each reaction performed in duplicate. The reaction mixture consisted of 2 µl of cDNA, 8 µl of RealQ Plus 2x Master Mix (Ampliqon, Denmark), 0.5 µl of each forward and reverse primer, and 9 µl of nuclease-free water.

The thermal cycling protocol for Real-Time PCR was as follows: an initial denaturation step at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, primer annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds. A melting curve analysis was subsequently performed to verify the specificity of the amplification.

*The relative expression levels of the target genes were calculated using the **2–ΔΔCT (Livak)** method.*

2.6 Statistical Analysis

The Kolmogorov-Smirnov (KS) test was used to determine the normality of data distribution, and Levene's test was used to check the homogeneity of variances. One-way analysis of variance and LSD post-hoc test were used to determine significant differences between groups. Data analysis was performed using SPSS software (version 23), with a significance level set at $P \leq 0.05$.

3 Results

The initial, final, and weight change values of the rats in the different groups are presented in Table 2. One-way analysis of variance showed no significant differences in initial weights among the groups. Additionally, final weights and weight changes (final weight − initial weight) showed no statistically significant differences among the groups, indicating baseline homogeneity between the groups.

Table 2. Values (Mean ± Standard Deviation) of Subjects' Weights in the Different Study Groups

NAFLD + High-intensity interval training	NAFLD control	Healthy control	Variable group
242/80±14/92	17/88±262	11/2±261/4	**initial weight(gr)**
315/80±25/52	19/1±335.02	13/28±316	**final weight(gr)**
73±12	73/20±22/15	22/02±54/60	**Weight changes (gr)**

3.1 Gene Expression Results

The results of one-way ANOVA (Table 3) indicated no significant differences in LECT2 gene expression among the experimental groups (F_{obs}=1.02 , P =.4) Similarly, relative expression of FGF21 was higher in the NAFLD control group compared to the healthy control group, though this difference was not statistically significant. Furthermore, no significant differences were observed among the experimental groups (F_{obs} = 0.59, P = 0.5). Regarding follistatin gene expression, the results also showed no significant differences between the groups (F_{obs}= 0.8, P = 0.4).

Table 3. Relative Expression (Mean ± Standard Deviation) of Genes in the Liver Tissue of the Study Groups

NAFLD + High-intensity interval training	NAFLD control	Healthy control	Variable group
2/68±./73	2/55±./82	2/06±./62	**LECT 2**
4/28±./48	4/56±1/1	3/51±./7	**FGF 21**
8/70±1/4	7/49±1/2	8/25±1/3	**follistatin**

4 Discussion

The aim of this research was to investigate the effect of high-intensity interval training on the gene expression of hepatokines in rats with non-alcoholic fatty liver disease. The results showed that none of the studied genes exhibited statistically significant differences among the groups after 8 weeks of training.

Leukocyte cell-derived chemotaxin 2 (LECT2) is a hepatic secretory protein initially identified as a neutrophil chemotactic factor (24). Studies have shown that its plasma levels increase in obesity, type 2 diabetes, and non-alcoholic fatty liver disease (9,10). Laboratory studies have also demonstrated that LECT2 induces inflammation in vascular endothelium and directly inhibits insulin signaling in C2C12 myotubes and 3T3-L1 adipocytes (18). Lan et al (2014) showed that LECT2 expression in the liver and its circulating concentration in mice are negatively regulated by the AMPK enzyme (11). Willis et al. (2020) and Chikamoto et al. (2016) also showed that LECT2 increases after a period of high-fat diet feeding (9,22). This is consistent with reduced AMPK expression and phosphorylation under satiety and high-calorie feeding conditions, clearly indicating an inverse relationship between LECT2 expression and AMPK phosphorylation in the liver (11). Sargeant et al in 2018 showed that 60 minutes of moderate-intensity treadmill exercise had no effect on circulating LECT2 concentrations up to 6 hours post-exercise in lean and overweight men (25). In the study by Willis et al (2018), plasma LECT2 levels remained unchanged after a single bout of intense and moderate exercise in healthy men (18). In the present study, no significant change in LECT2 gene expression was observed, which aligns with the findings of these human studies. One possible reason for this response is the negative regulation of LECT2 by AMPK and its increased activity after exercise, which may prevent significant changes in gene expression.

FGF21 is a hepatokine primarily secreted from the liver and plays a role in regulating fat and glucose metabolism in various tissues (8). Despite the numerous beneficial effects of FGF21 on glucose and lipid homeostasis and insulin sensitivity in animal models, circulating FGF21 levels increase in humans and animals with diet- or genetically-induced obesity, indicating FGF21 resistance in obesity (25,26). Plasma FGF21 levels also increase in patients with metabolic syndrome, type 2 diabetes, and non-alcoholic fatty liver disease (26). The data from the present study also showed that the relative expression of the FGF21 gene was higher in the diseased control group compared to the healthy group, although this difference was not statistically significant. Regarding the effect of exercise training on improving insulin resistance and its relationship with FGF21, the results are highly contradictory. Some studies report increases (27), some decreases (28,29), and others no changes (26,30). The results of the present study showed that FGF21 gene expression was higher in the fatty liver control group compared to the healthy control, but the difference was

not statistically significant. Additionally, HIIT training had no significant effect on this gene, which aligns with some animal and human studies (26,30). This varied response is likely due to differences in intensity, duration, type of exercise, and animal or human models.

Follistatin is a glycosylated plasma protein that is a member of the TGFβ family (5). It has recently been reported that follistatin is expressed not only in the liver but also in skeletal muscles and white and brown adipose tissues (31,32). Its plasma levels in obese individuals or those with metabolic disorders have been reported variably, and the reasons for these differences are still unclear (25). Studies have shown that aerobic exercises may reduce hepatic follistatin expression or have no effect (20). In the present study, no significant differences were observed among the groups. Given the limited and contradictory results available, further research is needed to investigate potential mechanisms and the effect of exercise intensity on follistatin.

5 Conclusion

Overall, the results of the present study showed that interval training had no effect on the gene expression of FGF21, follistatin, and LECT2 in the liver tissue of rats with fatty liver. These findings suggest that the effect of exercise on hepatokines may require longer protocols, higher intensity, or a combination of exercise and dietary changes. Additionally, for a better understanding of the interactions between these hepatokines and their roles in response to exercise and other metabolic disorders, future studies with human and animal samples, longer training periods, and simultaneous measurement of protein and mRNA are recommended.

Ethical Considerations

All care procedures and ethical principles were observed in accordance with the guidelines for the use and care of laboratory animals and approved by the Ethics Committee of the University of Mazandaran. Additionally, this project was reviewed by the Research Ethics Committee of the University of Mazandaran and approved with the code IR.UMZ.REC.1399.018.

Acknowledgments

I hereby sincerely thank my supervisor, Dr. Ali Younesian, for his tireless efforts, and also express my utmost gratitude to Dr. Elahe Talebi Garakani for her attention and precision in providing consultation.

REFERENCES

[1] Cho J, Kim S, Lee S, Kang H. Effect of training intensity on nonalcoholic fatty liver disease. Med Sci Sports Exerc. 2015;47(8):1624–34.

[2] Liu J, Xu Y, Hu Y, Wang G. The role of fibroblast growth factor 21 in the pathogenesis of non-alcoholic fatty liver disease and implications for therapy. Metabolism. 2015 Mar 1;64(3):380–90.

[3] Lebensztejn DM, Flisiak-Jackiewicz M, Bialokoz-Kalinowska I, Bobrus-Chociej A, Kowalska I. Hepatokines and non-alcoholic fatty liver disease. Vol. 63, Acta Biochimica Polonica. Polskie Towarzystwo Biochemiczne; 2016. p. 459–67.

[4] Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. Vol. 75, Cellular and Molecular Life Sciences. Birkhauser Verlag AG; 2018. p. 3313–27.

[5] Ennequin G, Sirvent P, Whitham M. Role of exercise-induced hepatokines in metabolic disorders. Am J Physiol Endocrinol Metab [Internet]. 2019;317:11–24. Available from: http://www.ajpendo.org

[6] Meex RCR, Watt MJ. Hepatokines: Linking nonalcoholic fatty liver disease and insulin resistance. Vol. 13, Nature Reviews Endocrinology. Nature Publishing Group; 2017. p. 509–20.

[7] Stefan N, Häring HU. The role of hepatokines in metabolism. Vol. 9, Nature Reviews Endocrinology. 2013. p. 144–52.

[8] Inagaki T. Research Perspectives on the Regulation and Physiological Functions of FGF21 and its Association with NAFLD. Vol. 6, Frontiers in Endocrinology. Frontiers Media S.A.; 2015.
[9] Chikamoto K, Misu H, Takayama H, Kikuchi A, Ishii KA, Lan F, et al. Rapid response of the steatosis-sensing hepatokine LECT2 during diet-induced weight cycling in mice. Biochem Biophys Res Commun. 2016;478(3):1310–6.
[10] Zhang Z, Zeng H, Lin J, Hu Y, Yang R, Sun J, et al. Circulating LECT2 levels in newly diagnosed type 2 diabetes mellitus and their association with metabolic parameters. Medicine (United States). 2018 Apr 1;97(15).
[11] Lan F, Misu H, Chikamoto K, Takayama H, Kikuchi A, Mohri K, et al. LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance. Diabetes. 2014;63(5):1649–64.
[12] Hussien NI, El-Kerdasy HI, El-Tantawy Ibrahim M. Downloaded from www.nrcresearchpress.com by TUFTS UNIV LIBRARY on 07/31/17 Can [Internet]. J. Physiol. Pharmacol. Downloaded from www.nrcresearchpress.com by TUFTS UNIV LIBRARY on. Available from: www.nrcresearchpress.com
[13] Hansen JS, Rutti S, Arous C, Clemmesen JO, Secher NH, Drescher A, et al. Circulating follistatin is liver-derived and regulated by the glucagon-to-insulin ratio. Journal of Clinical Endocrinology and Metabolism. 2016 Feb 1;101(2):550–60.
[14] Hallsworth K, Fattakhova G, Hollingsworth KG, Thoma C, Moore S, Taylor R, et al. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. Gut. 2011 Sep;60(9):1278–83.
[15] Hallsworth K, Thoma C, Hollingsworth KG, Cassidy S, Anstee QM, Day CP, et al. Modified high-intensity interval training reduces liver fat and improves cardiac function in non-alcoholic fatty liver disease: A randomized controlled trial. Clin Sci. 2015;129(12):1097–105.
[16] Oh S, So R, Shida T, Matsuo T, Kim B, Akiyama K, et al. High-intensity aerobic exercise improves both hepatic fat content and stiffness in sedentary obese men with nonalcoholic fatty liver disease. Sci Rep. 2017 Feb 22;7.
[17] Hayati M, Fatahi F, Abdolhossein ;, Kalani T. Aerobic-resistance Training and Caloric Restriction on Hepatokine Factors Alterations in Obese Women with Non-alcoholic Fatty Liver Disease. Women Health Bull. 2025;12(3):170–9.
[18] Willis SA, Sargeant JA, Thackray AE, Yates T, Stensel DJ, Aithal GP, et al. Effect of exercise intensity on circulating hepatokine concentrations in healthy men. Applied Physiology, Nutrition and Metabolism. 2019;44(10):1065–72.
[19] Mounesan A, Nourzad F. Journal of Exercise & Organ Cross Talk. 2023;3(4):208–
[20] Silva RN, Bueno PG, Avó LRS, Nonaka KO, Selistre-Araújo HS, Leal AMO. Effect of physical training on liver expression of activin A and follistatin in a nonalcoholic fatty liver disease model in rats. Brazilian Journal of Medical and Biological Research. 2014;47(9):746–52.
[21] Pang Wen C, Pui Man Wai J, Kuang Tsai M, Chen Yang Y, Yuan David Cheng T, Lee MC, et al. Articles Minimum amount of physical activity for reduced mortality and extended life expectancy: a prospective cohort study. www.thelancet.com [Internet]. 2011;378:1244–53. Available from: www.thelancet.com
[22] Willis SA, Sargeant JA, Yates T, Takamura T, Takayama H, Gupta V, et al. Acute hyperenergetic, high-Fat feeding increases circulating FGF21, LECT2, and fetuin - A in healthy men. Journal of Nutrition. 2020 May 1;150(5):1076–85.
[23] Kolahdouzi S, Talebi-Garakani E, Hamidian G, Safarzade A. Exercise training prevents high-fat diet-induced adipose tissue remodeling by promoting capillary density and macrophage polarization. Life Sci. 2019 Mar 1;220:32–43.
[24] Kikuchi A, Takayama H, Tsugane H, Shiba K, Chikamoto K, Yamamoto T, et al. Plasma half-life and tissue distribution of leukocyte cell-derived chemotaxin 2 in mice. Sci Rep. 2020 Dec 1;10(1).
[25] Sargeant JA, Aithal GP, Takamura T, Misu H, Douglas JA, Turner MC, et al. The influence of adiposity and acute exercise on circulating hepatokines in normal 1 weight and overweight/obese men 2 [Internet]. Available from: www.nrcresearchpress.com
[26] Besse-Patin A, Montastier E, Vinel C, Castan-Laurell I, Louche K, Dray C, et al. Effect of endurance training on skeletal muscle myokine expression in obese men: Identification of apelin as a novel myokine. Int J Obes. 2014;38(5):707–13.
[27] Cuevas-Ramos D, Almeda-Valdés P, Meza-Arana CE, Brito-Córdova G, Gómez-Pérez FJ, Mehta R, et al. Exercise increases serum fibroblast growth factor 21 (FGF21) levels. PLoS One. 2012 May 31;7(5).

[28] Taniguchi H, Tanisawa K, Sun X, Kubo T, Higuchi M. Endurance exercise reduces hepatic fat content and serum fibroblast growth factor 21 levels in elderly men. Journal of Clinical Endocrinology and Metabolism. 2016 Jan 1;101(1):191–8.

[29] Azali Alamdari K, Khalafi M. THE EFFECTS OF HIGH INTENSITY INTERVAL TRAINING ON SERUM LEVELS OF FGF21 AND INSULIN RESISTANCE IN OBESE MEN. Iranian Journal of Diabetes and Metabolism [Internet]. 2019 [cited 2025 Oct 27];18(1):41–8. Available from: https://ijdld.tums.ac.ir/article-1-5735-en.html

[30] Kruse R, Vienberg SG, Vind BF, Andersen B, Højlund K. Effects of insulin and exercise training on FGF21, its receptors and target genes in obesity and type 2 diabetes. Diabetologia. 2017 Oct 1;60(10):2042–51.

[31] Braga M, Reddy ST, Vergnes L, Pervin S, Grijalva V, Stout D, et al. Follistatin promotes adipocyte differentiation, browning, and energy metabolism. J Lipid Res. 2014;55(3):375–84.

[32] Hansen J, Brandt C, Nielsen AR, Hojman P, Whitham M, Febbraio MA, et al. Exercise induces a marked increase in plasma follistatin: Evidence that follistatin is a contraction-induced hepatokine. Endocrinology. 2011 Jan;152(1):164–71.