Research Article

The effects of high-intensity interval training on the expression of interleukin-10 and STAT3 genes in the intestinal tissue of rats affected by hepatic steatosis

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Abstract

Hepatic steatosis is increasingly being recognized as an important pathological feature of disease that commonly reported in patients with inflammatory bowel disease. This study aimed to investigate the effects of High-Intensity Interval Training (HIIT) on the expression of interleukin-10 and Signal transducer and activator of transcription 3 (STAT3) genes in intestinal tissue in an animal model of fatty liver. In this experimental study, 24 rats (weighing 200-250 gr) were selected and randomly divided into 3 groups including healthy control, fatty liver, and fatty liver + HIIT, groups. In order to induce fatty liver, oral tetracycline 140 mg/kg/day in 2 mL of water in form of a solution was given to the rats by gavage for 7 days. HIIT exercise program performed on treadmill five sessions per week for 5 weeks. Data were analyzed by one-way ANOVA and Tukey post hoc tests. $P<0.05$ was considered significant. The results showed that IL10 gene expression in HIIT groups was significantly lower than in the fatty liver group ($p<0.0001$). Also, the expression of the STAT3 gene in intestinal tissue was significantly upper in HIIT groups than that in the fatty liver group ($p<0.0001$). Regulation of IL-10 and STAT3 gene expression in fatty liver-induced adipose tissue can be modulated by HIIT exercise. Therefore, intense interval training can be considered as a non-pharmacological strategy in the treatment of fatty liver.

Key Words: **High intensity interval training, Hepatic steatosis, STAT3, Interleukin-10**

Introduction

Steatosis or Non-Alcoholic Fatty Liver Disease (NAFLD) is one of the most widespread liver diseases all over the world and in all age groups. According to the latest reports, NAFLD is seen in 17-46% of the world population (Magrì et al., 2019). In fact, steatosis or the non-alcoholic accumulation of fat in the liver is recognized as one of the first liver diseases that is accompanied by inflammation and can lead to alcoholic liver disease. Severe damage to the liver can lead to the onset and continuity of inflammation processes that combined with a set of processes and related paths, lead to the deposition of fibrous tissue (Poynard et al., 2002). NAFLD is observed in 33.6% of patients with IBD. Furthermore, in patients with severe IBD, NAFLD is reported in sonography. Moreover, research has shown that IBD in NAFLD is accompanied by an increase in pro-inflammatory and anti-inflammatory cytokines (Nicoletti et al., 2019). IBD is associated with genetic factors in innate and adaptive immunity and the genes related to adaptive immunity include interleukin IL-10, IL-12, IL-23, keeping the equilibrium between inflammatory and non-inflammatory cytokines (Bonen & Cho, 2003). There is a consensus that IL-10 is an oppressor of the immune system with so strong effects that its activity should be precisely regulated. Otherwise, the effect would include serious illnesses related to the overproduction of IL 10 and unwanted oppression of the immune system. On the other hand, the underproduction or lack of IL 10 leads to the unceasing activation of immunity such as rheumatoid arthritis and severe IBD.

IL 19 is almost produced by all leukocytes. For instance, the IL 10 produces by T cells is needed for controlling severe inflammations, but useless for acute inflammation. For anti-inflammatory responses, the link between IL 10 to IL 10R leads to the activity of L10/JAK1/STAT3 pathway (Roers et al., 2004). Lower signaling path of the IL-10 family is a signal transducer and activator of transcription 3 (STAT3). In fact, an increase in IL-10 stimulates the activation of the STAT3 pathway. Both IL-10 and STAT3 are necessary for anti-inflammatory responses

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and cannot be replaced by any other cytokine or transcription factor. Signal transducer and activator of transcription 3 (STAT3) are members of the family of cytoplasmic proteins taking part in the natural cellular response to cytokines and growth factors as transcription factors. STAT3 balances the various physiological functions such as cell survival, regulation of cell cycle and angiogenesis, through the regulation of genetic expression, and the activation of its producer is related to a couple of epithelial cancers in humans (Krishnamurthy et al., 2009).

Numerous drug therapies have been mentioned for fatty liver, which are not recommended because of their side effects, high costs and weight gain. However, dietary correction and lifestyle change through exercise have been accepted as standard treatment. Studies have been shown that exercise has anti-inflammatory effects and many studies have been reported an increase in the production of IL-10 after exercise, although some researchers have not reported this increase to be meaningful (Shafieerad et al., 2018). It has also been shown that exercise through phosphorylation STST3 can lead to an increase in its activity (Calbet et al., 2011). Lots of researchers have studied the effects of physical activity on fatty liver, yet most of these researches have been about traditional exercise with low or medium intensity (Vickers, 2017). Therefore, the effects of the level and intensity of exercise have not clearly been researched. Based on the lack of studies on High-Intensity Interval Training, it seems like these type of exercise can have different effects on fatty liver. In a study, the patients who participated in high-intensity activities showed less signs of liver fibrosis compared to those participated in medium-intensity exercise (Keating et al., 2015). Considering the link between this illness and inflammation, this research aimed to measure the effects of different types of exercise (HIIT) on the expression of genes related to anti-inflammatory factor (IL-10, STAT3) in the intestinal tissue of the animal model of fatty liver.

Materials and Methods

Animals

This research is an experimental and practical study. In this research, the studied population included male Wistar rats in two groups of healthy rats and rats with fatty liver (steatosis). The samples were comprised of 24 rats weighting between 200-250 grams, randomly distributed in 3 groups each with 8 rats. These groups were the healthy control group, the fatty liver (steatosis) group and the fatty liver (steatosis) group with HIIT exercise.

Fatty liver induction

Tetracycline was administered orally at a dose of 140 mg per kilogram of body weight (as a solution in 2 mL of water) to rats by gavage for 7 days. Fatty liver (steatosis) was confirmed by measuring liver enzymes and hematoxylin-eosin staining (Shabana et al., 2012). In this study, an increase in liver enzymes was observed. The advantages of creating this type of fatty liver model were factors the similarity of complications in humans and animals and the control of laboratory conditions and investigation at the same time.

Exercise protocols

In order to familiarize and create adaptation to the main training conditions, the rats were placed on the treadmill for three sessions in the first week at a speed of 7 to 10 meters per minute for 5 to 10 minutes and were forced to exercise. Then, the HIIT training program was performed for 5 weeks and 5 sessions per week according to Table 2 and the main principles of intense interval training (Kalaki-Jouybari et al., 2020). The warm-up and cool-down stages were also performed for 4 to 8 minutes with 40% of the maximum running intensity (Table 1).

Laboratory mesurments

48 hours after the last training session (10-12 hours fasting), the study rats per group were intraperitoneally injected with a mixture of ketamine 10% at a dose of 50 mg/kg and xylazine 2% at a dose of 10 mg/kg. The end of the small intestinal tissue (ilium) of rats was then sampled; after washing in physiological serum, it was immersed in 1.8 microtubes, containing RNAlaterTM¹ fluid with a

Table 1. HIIT program.

Week	Warm-up Speed (Meters Per Minute)	Fast Rotation Number	Fast Rotation Speed (Meters Per Minute)	Number of Slow Rotations	Slow Rotation Speed (Meters Per Minute)
First	4	5 intervals 2-minute	16-20	5 intervals 1-minute	10
Second	5	5 intervals 2-minute	21-25	5 intervals 1-minute	11
Third	6	5 intervals 2-minute	26-30	5 intervals 1-minute	12
Fourth	7	5 intervals 2-minute	31-35	5 intervals 1-minute	13
Fifth	8	5 intervals 2-minute	36-40	5 intervals 1-minute	14

Table 2. The sequence of used primers for the studied variables.

Genes name	Primer sequences
IL-10	Forward: CTGCTCTTACTGGCTGGAGT
	Reverse: TGGGAAGTGGGTGCAGTTAT
STAT3	Forward: CCCATAGTGAGCCCTTGGAA
	Reverse: TGCAGTGACCAGGACAGAAT
GAPDH	Forward: CAAGTTCAAGGGCACAGTCA
	Reverse: CCCCATTTGATGTTAGCGGG

testing. The gene expression of the desired factors was measured from intestinal tissue by Real-time-PCR technique. After quantification, the gene expression values were analyzed by the formula $2^{-\Delta\Delta ct}$. The PCR reaction was performed using (Applied Biosystems) PCR master mix and SYBR Green in the device (Applied Biosystems, Sequence Detection Systems. Foster City, CA) ABI Step One according to the manufacturer's protocol. The sequence of used primers is listed in Table 2.

Statistical analysis

The Kolmogorov-Smirnov test was used to ensure the normal distribution of the data and the Levine test was used to ensure the homogeneity of the variances. Descriptive statistics were used to describe the data and graphs, and one-way ANOVA was used to compare the changes between groups in the studied variables. Tukey's post hoc test was used to examine the differences between groups. The significant level was also considered P≥0.05. All the statistical studies were done using SPSS/Win version 20 software.

Results

Data analysis showed that IL-10 gene expression in the intestinal tissue in the steatosis animal model was significantly higher than the healthy group (p<0.0001). While compared to the control group, the value of IL-10 gene expression in the HIIT group increased and non-significant, but in the HIIT + steatosis group it increased significantly (p=0.0075). Compared to the steatosis group, the HIIT and HIIT + steatosis groups decreased IL-10 gene levels (p<0.0001). Compared to the HIIT group, IL-10 gene expression levels increased in the HIIT + steatosis group (p=0.01) (Figure 1)

Furthermore, data analysis showed that the expression of intestinal tissue STAT3 gene in the animal model of steatosis was significantly lower than the healthy group (p<0.05). While compared to the control group, the value of STAT3 gene expression in the HIIT and HIIT + steatosis groups increased significantly (p<0.01). Compared to steatosis group, HIIT and HIIT + steatosis groups increased STAT3 gene levels (p<0.0001). Compared to the HIIT group, the intestinal STAT3 gene expression levels were decreased in the HIIT + steatosis group (p=0.01). (Figure 2)

Discussion

As the results of this research shown that the expression of IL 10 gene in the intestinal tissue of rats in the fatty liver group increased meaningfully compared to other groups. These results showed that fatty liver caused inflammation in intestinal tissue, which then can be balanced by the increase in anti-inflammatory

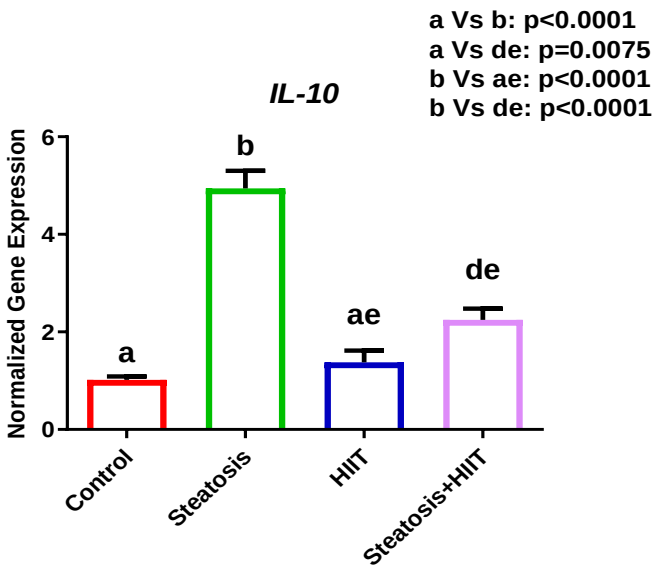
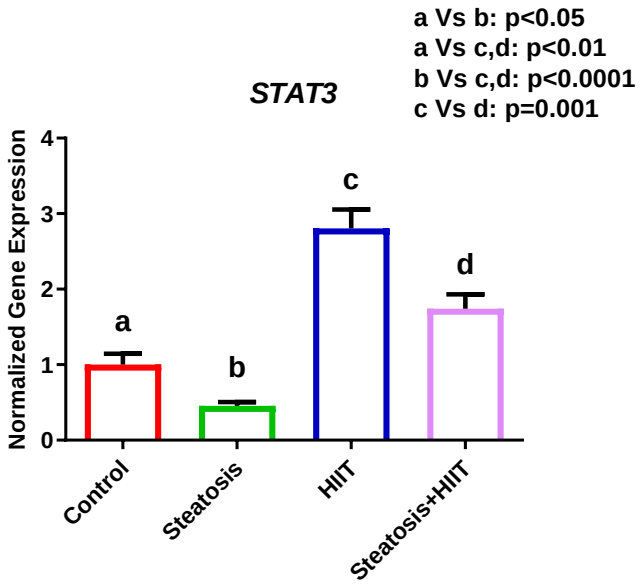


Figure 1. Gene expression of IL-10 at different groups (steatosis and exercise). Data were show as mean±SD. HIIT: High Intensity Interval Training.



Fgure 2. Gene expression of STAT3 at different groups (steatosis and exercise). Data were show as mean±SD. HIIT: High Intensity Interval Training.

factors like IL-10. It has been reported that IL-10 is discharged in response to the produced inflammation. The extra increase in the anti-inflammatory IL-10 cytokine faces the immune system a challenge to form an inflammatory response, leading to injection. Too much intense exercise follows the same mechanism, which is not in line with the current study (Shaw et al., 2018).

Studies have shown that the anti-inflammatory effects of physical exercise are first mediated by IL-10 (Batista Júnior et al., 2009). In the same way, some studies, in line with the current study, have shown the role of HIIT exercise on the regulation of the expression of IL 10 gene. Several studies have shown the effects of high-intensity exercise on the decrease of fat in the liver of humans and animals, which could help in the treatment of fatty liver patients (Hallsworth et al., 2015), since high-intensity exercises had more effect on the decrease of steatosis and liver fibrosis compared to medium-intensity training. Generally, molecular mechanism shows that physical training causes the regulation of the IL-10 in the T cell pathway via Th2 by negative regulation of the activities of NF-kB (Nicklas et al., 2005). On the other hand, balancing the cytokine discrete from Th1 and Th2 through regular exercise has been proven. Contrary to the results of current study, some studies have shown that very high-intensity interval training has caused meaningful increase in IL-10 (Vahdat et al., 2018). Furthermore, the study done by Ranjbar et. al. has shown that IL-10 levels after 8 weeks of HIIT exercise in men with diabetes type 2 have not considerably changed. Thus, it seems like the anti-inflammatory effects of exercise depends on its intensity and duration. Dorneles et. al. reported that the inflammatory response to be dependent on the intensity of the exercise, since they have shown an increase in IL 10 in fat people after high-intensity training (Dorneles et al., 2016). Moreover, the results of the current study have indicated that the expression of STAT3 gene in the liver tissue of rate in the HIIIT group increased considerably compared to all the other groups. IL-10 enables the effective activation of signaling pathway IL-10/JAK1/STAT3, and phosphorylation STAT3 is necessary for this. Furthermore, mutation of IL-10 gene or its receiver (IL-10RA) causes a disruption in the activation of STAT3. The incomplete regulation and too much increase in the anti-inflammatory factors increase the risk of infection, which is in agreement with the results of the current study. Probably, the overexpression of IL-10 has caused a decrease in the activities of STAT3 in the fatty liver group (Glocker et al., 2009). As was said before, exercise causes an increase in the activation of STAT3 through its phosphorylation. This is also consistent with the results of the current study. Studies have shown the alignment of the effects of exercise with an increase in the activities of STAT3 (Rodrigues Brandao-Rangel et al., 2017). Jia et al. has shown that interval training caused an increase in the activities of STAT3 in male rats, that is in line with the results of the current study (Jia et al., 2018).

Conclusion

The effects of the anti-inflammatory IL-10 cytokine are regulatory and both over- and underproduction of it caused disruptions of the functioning of immunity. Based on the results of this research, High-intensity Interval Training balanced the expression of anti-inflammatory cytokine genes through cellular mechanism. Therefore, HIIT can probably help in the treatment of fatty liver.

What is already known on this subject?

Hepatic steatosis is increasingly being recognized as an important pathological feature of disease that commonly reported in patients with inflammatory bowel disease.

What this study adds?

High-intensity Interval Training balanced the expression of anti-inflammatory cytokine genes through cellular mechanism. Therefore, HIIT can probably help in the treatment of fatty liver.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research has been done according to the ethical code at the Health and Exercise Physiology Research Institute of the Baqiyatallah University of Medical Sciences.

Informed consent Animal study.

Author contributions

Conceptualization: E.K, F.R.; Methodology: E.K, F.R.; Software: E.K, F.R.; Validation: E.K, F.R.; Formal analysis: E.K, F.R.; Investigation: E.K, F.R.; Resources: E.K, F.R.; Data curation: E.K, F.R.; Writing - original draft: E.K, F.R.; Writing - review & editing: E.K, F.R.; Visualization: E.K, F.R.; Supervision: F.R; Project administration: E.K, F.R.; Funding acquisition: F.R.

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