Research Article

The effect of high-intensity interval training on IL-22 and STAT3 gene expression of liver tissue in steatosis animal model

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Abstract

Inflammation is a major component of almost all acute and chronic liver disorders, including non-alcoholic fatty liver disease. This study aimed to investigate the effect of high-intensity interval training on IL-22 and STAT3 gene expression of liver tissue in steatosis animal model. In this experimental study, 32 rats (weighing 200-250 gr) were selected and randomly divided into 4 groups including healthy control, fatty liver, HIIT and fatty liver + HIIT group. Rats were infected with fatty liver by oral tetracycline at a dose of 140 mg/kg (soluble in 2 ml of water) for 7 days. The HIIT exercise program performed on treadmill five sessions per week for 5 weeks. The IL-22 and STAT3 gene expressions in the liver tissue of samples were measured by Real Time PCR. Data were analyzed by One-way ANOVA and Tukey post hoc tests at significance level P <0.05. The results showed that the gene expression of IL-22 in liver tissue in HIIT group and fatty liver + HIIT was significantly lower than that in the fatty liver group (P = 0.001). Also, the gene expression of STAT3 in liver tissue in HIIT group and fatty liver + HIIT was significantly higher than that in the fatty liver group (P = 0.001). According to the results, the HIIT training program seems to help improve the liver steatosis.

Key Words: **High-intensity interval training, Fatty liver, IL-22, STAT3, Rats**

Introduction

Inflammation is considered to be a major component in all acute and chronic cases, including fatty liver disorders such as non-alcoholic fatty liver disease (NAFLD) (Ferrante et al., 2000). Immune mediators, especially cytokines, have been shown to be able to control many of the main features of the disease, including acute health failure, acute phase response, steatosis, cholestasis, hypergammaglobulinemia, and the development of fibrosis. In most tissues, including cases, in physiological conditions, cytokines also cause tissue damage (Niederreiter & Tilg, 2018). Factors such as non-alcoholic fatty acids play an important role in mitochondrial performance,fatty acids toxicity and immune systems in liver damages such as non-alcoholic fatty liver , but the mechanisms involved in identifying the factors that promote steatosis in damaged lesions have remained unknown (Carmo et al., 2017). Recent studies support an important association between interleukin-22 (IL-22)/phosphorylated signal transducer and activator of transcription 3 (STAT3) with nonalcoholic fatty liver disease and evidence suggests that IL-22 may represent a potential factor in treating fatty liver (Wu et al., 2020).

IL-22 is an inflammatory cytokine that structurally belongs to the interleukin 10 family (Dudakov et al., 2015). This cytokine plays an important role in promoting the immune system and improving liver tissue. It has been found that IL-22, unlike many cytokines, does not directly regulate the immune system, but binds and donates to cells in the external barriers of the body, such as skin, digestive system tissue, pancreatic cells, liver, kidney, and joints (Carmo et al., 2017). IL-22 reduces tissue inflammation and, with its protective function, improves and defends the host tissue (Yang et al., 2010). In addition, studies have shown that treatment with IL-22 reduces liver damage, fatty liver and oxidative stress (Carmo et al., 2017). In contrast, IL-22 is also defined as a pro-inflammatory cytokine. Oxidative stress caused by liver steatosis and high fat increases the expression of IL-22 as a pro-inflammatory (Mo et al., 2018). The protective function of IL-22 in liver injury patients seems to be

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mediated by a mechanism involving STAT3 activation (Ki et al., 2010). Through binding to its specific heterodimer receptor on the cell surface, this cytokine causes the phosphorylation of the inflammatory mediators STAT1, ATAT3 and STAT5 and finally leads to the activation of the signal transduction and transcription activator (STAT) pathway - Janus kinase (JAK). Also, the activity of IL-22 in extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK) and mitogen-activated kinase (MAPK) pathways has also been observed (Ziesché et al., 2007). Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that is activated by many cytokines and growth factors and plays a key role in cell survival, proliferation and differentiation, and STAT3 activation has been identified in the unhealthy liver of all animal and human models (Zhao et al., 2021).

Changes in lifestyle, such as physical activity and diet, may provide important clinical benefits for improving inflammation in the long term (Beavers & Nicklas, 2011). In this regard, it has been reported that physical exercise improves the metabolic, oxidant and inflammatory status, and preliminary evidence suggests the effect of sports training as an effective and multifaceted factor on intracellular messenger pathways that can be effective in reducing fat tissue, reducing inflammatory substances (Woo & O'Brien, 2012). Ramos et al. (2020) investigated the effect of interval and continuous different training density on IL-22 in people with metabolic syndrome for 16 weeks.The results showed no significant difference between the effect of different density of exercises on IL-22 concentration (Ramos et al., 2020). Rangel et al. (2017) investigated the effects of aerobic exercise on STAT3 in animal models (6-week-old rats) with chronic pulmonary obstruction, which showed a decrease in STAT3 and an increase in IL-10 in the blood serum of the exercise group. These results indicate that moderate-intensity aerobic exercise inactivates STAT3 (Rangel et al., 2017).In contrast, Zhao et al. (2011) reported that STAT3 in the hypothalamus of Wistar rats increased in response to 9 weeks of endurance exercise (Zhao et al., 2011) Today, HIIT is used instead of endurance training due to its attractiveness, variety, and greater metabolic compatibility. Some researchers have suggested that HIIT training is more effective than endurance training for weight loss and fat loss (Gibala et al., 2012), and a 7-10% reduction in body weight can significantly reduce hepatic steatosis (Keating et al., 2015), but information regarding the effect of HIIT training on IL-22 and STAT3 variables was not observed. Perhaps due to the variation in the intensity and duration of exercise, HIIT training can have more beneficial changes than other sports training on determinants in improving liver steatosis.Thus, the present study aims to investigate the effect of intense intermittent training on the expression of IL-22 and STAT3 genes in liver tissue is an steatosis animal model.

Materials and Methods

Animal

The current research is of fundamental type with experimental method. The statistical population consisted of all male Wistar rats from the animal house of Pasteur Institute, of which 32 male Wistar rats in the weight range of 200-250 grams were randomly selected to participate in the study. Male Wistar rats were randomly divided into four groups of eight including healthy control group, fatty liver (steatosis), HIIT, and fatty liver + HIIT. The rats were kept in the Physiology Laboratory of Baqiyatullah University of Medical Sciences at 20-23 degrees Celsius and a light-dark cycle of 12 hours and relative humidity of 50% with standard food and water.

Fatty liver

Tetracycline was administered orally at a dose of 140 mg per kg 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 (Shabana et al., 2012).

Intense interval training protocol

At first, the rats were placed on the treadmill for 5-10 minutes for 3-5 sessions per week in order to familiarize them with the main training. Then, the intense interval training program (5 sessions per week) and running on a treadmill for 5 weeks were carried out based on the general principles of high intensity interval training (HIIT) (Kalaki-Jouybari et al., 2020). In this protocol, the HIIT training program included warm-up and cool-down phases for 4 to 8 minutes with an intensity of 40% of maximum running. The protocol for the implementation of the exercise sessions is given in Table 1.

Table 1. Intense interval training on treadmill

Weeks	Fast interval Set/min/m-min	Slow interval Set/min/m-min
1	5/2/16-20	5/1/10
2	5/2/21-25	5/1/11
3	5/2/26-30	5/1/12
4	5/2/31-35	5/1/13
5	5/2/36-40	5/1/14

Tissue sampling and measurement of laboratory variables

48 hours after the last training session (10 to 12 hours of fasting), the rats under study in each group were injected intraperitoneally with a mixture of 10% ketamine at a dose of 50 mg/kg and 2% xylazine and at a dose of 10 mg/kg fainted. Then, the liver tissue of the rats was sampled and after being washed in physiological serum, it was immersed in 1.8 microtubes containing 20% RNAlaterTM liquid and transferred to the laboratory for genetic tests. RNA was extracted from liver tissue by the Rneasy protect mini kit (QIAGEN) according to the company's instructions. 20 mg of tissue was chopped using a scalper and inserted into a microtube, and then RNA was extracted using the RNeasy Protect kit according to the instructions of the German manufacturer. After RNA extraction, to ensure the sufficient concentration of RNA in cDNA preparation, it was checked by a nanodrop device. IL-22 and STAT3 mRNA by RT-Real time PCR by Rotorgen 6000 system using One Step SYBR TAKARA kit from Takara company was determined according to the company's instructions. Melting curve analysis was performed at the end of the PCR cycle to determine the validity of the expected PCR product. The thermal cycle protocol used by Rotogen device in Real time-PCR included: 42° for 20 minutes, 95° for 2 minutes and 40 cycles with 94° for 10 seconds and 60° for 40 seconds. After the PCR step, in order to study the characteristics of the primers, temperatures from 50 to 99°C were used to prepare the melting curve.

Polymrasell RNA was used as a control gene to determine the expression of the studied genes and CTs related to the reactions were extracted and recorded by Real time-PCR machine software. The gene expression of the desired factors was measured from the liver tissue by Real time-PCR technique and after quantifying the expression values of IL-22 and STAT3 genes, it was analyzed with the formula $ct^{\Delta\Delta\text{-}2}$. The PCR reaction was performed using (Applied Biosystems) PCR master mix and SYBR Green in the ABI Step One (Applied Biosystems, Sequence Detection Systems, Foster City, CA) according to the manufacturer's protocol. The sequence of the primers used is listed in Table 2.

Table 1. Sequence of primers used in the variables of the study

Gene name	primers	sequence	Length
STAT3	Forward	GACCGACCCCAAGTACATCA	104 bp
	Reverse	CTGTATCTGCGCACTGGAAC	
IL-22	Forward	AGAGATTTGGTGCCACTATGAA	123 bp
	Reverse	ACCACTTGACATTATCGTTCCT	
GAPDH	Forward	CAAGTTCAAGGGCACAGTCA	104 bp
	Reverse	CCCCATTTGATGTTAGCGGG	

Statistical analysis

After the normality of the data was confirmed with the Shapiro-Wilk test, one-way analysis of variance was used for inter-group changes and Tukey's post hoc test to examine the differences between groups. All the statistical operations of the research were done using SPSS version 23 software, and the significance level of $P<0.05$ was considered.

Results

The results of the correlated t test show that there is a significant difference between the pre-test and post-test endostatin values of the EMS group (P=0.012), but there is no significant difference between the pre-test and post-test endostatin values of the control group (P=0.206). Also, the results of the independent t-test show that there is no significant difference between the two groups of the endostatin in post-test values (Figure 1).

Data analysis showed that there is a difference between the average expression of STAT3 gene in liver tissue in animal model

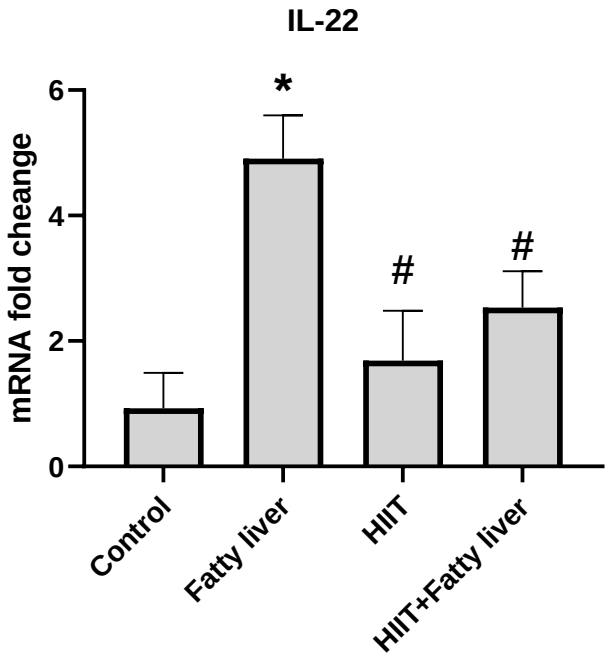

Figure 1. Changes in IL-22 gene expression in liver tissue in the steatosis animal model with 5 weeks of intense interval training in different groups. * Significant difference with the healthy control group; # significant difference with fatty liver group ($p \geq 0.05$).

of steatosis in different research groups (P=0.001). The results of Tukey's post hoc test showed that the expression of the STAT3 gene in the liver tissue in the fatty liver group was not significantly different from that in the healthy control group (P=0.158).The expression of the STAT3 gene in the liver tissue in the HIIT and fatty liver + HIIT groups compared to the fatty liver group was significantly higher (P=0.001),and the expression of STAT3 gene in the liver tissue was significantly higher in the HIIT group than in the fatty liver + HIIT group (P=0.005) (Fig. 2).

Discussion

The findings of the present research show that fatty liver was associated with increased IL-22 gene expression in the animal model of steatosis. Intense intermittent exercise led to a significant decrease in IL-22 gene expression in an steatosis animal model . The finding of the present study is consistent with the results of Alvarenga et al., who reported a decrease in IL-22 concentration after a 12-week exercise program (Alvarenga-Filho et al., 2016). The exact mechanism of IL-22 gene expression changes following sports training is not known, and it has been reported that IL-22 changes are affected by training intensity. In the same context, in the study of Ramos et al. (2020), although there was no statistically significant difference in IL-22 changes between exercise training groups, they stated that different exercise intensities might have opposite effects on IL-22 levels

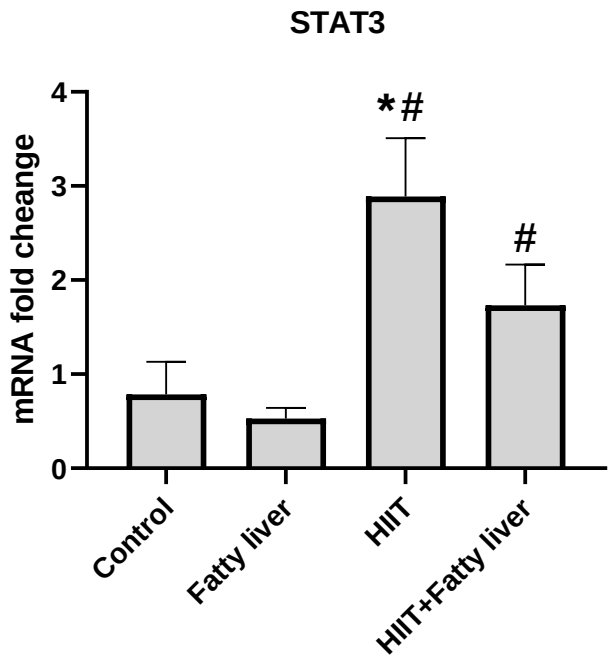

Figure 1. Changes in STAT3 gene expression in liver tissue in the steatosis animal model with 5 weeks of intense interval training in different groups. * Significant difference with the healthy control group; # significant difference with fatty liver group (p ≥ 0.05).

in blood circulation. It has been reported that the reduction of IL-22 can be partially caused by the direct ability of training intensity to improve the function of endothelial and pancreatic beta cells, which affect the changes of IL-22 (Ramos et al., 2015; Slentz et al., 2009), and in the present study, intense intermittent training was able to lead to a significant decrease in IL-22 gene expression. It is said that due to the severity of fatty liver disease and the need for greater immunity, exercise reduces IL-22, and another mechanism is that physical activity can reduce IL-22 by reducing insulin resistance (Slentz et al., 2009). Therefore, it is possible that HIIT exercises in the present study have contributed to the reduction of IL-22 gene expression through the reduction of insulin resistance, although insulin resistance was not measured in this study, which can be considered as a limitation. It has also been reported that HIIT improves mitochondrial function (Tjønna et al., 2008), so the improvement of mitochondrial function in tissues affected by HIIT may play a role in the regulation of IL-22 gene expression and activation (Dalmas et al., 2014). In a study conducted in patients with metabolic syndrome, a decrease in pro-inflammatory cytokines was reported in parallel with a favorable change in the anti-inflammatory cytokine IL-10, of which IL-22 is known to be a key member, following a period of HIIT exercise (Steckling et al., 2016).

Furthermore, a reduction in visceral adipose tissue occurs following HIIT exercise interventions, indicating a reduction in hypoxia-induced necrosis and the consequent release of pro-inflammatory cytokines (Pasarica et al., 2009). Therefore, according to the significant decrease in IL-22 gene expression, HIIT exercises may have contributed to the decrease in IL-22 gene expression through the effect on the above-mentioned factors. In contrast to the findings of the present research, it has been shown that HIIT leads to a non-significant increase in serum IL-22 in adults with metabolic syndrome, which may be due to factors such as the type of subjects, disease conditions, and location. The measurement is relevant. Another finding of the current research showed that intense intermittent training leads to a significant increase in the expression of the STAT3 gene in the liver tissue in a steatosis animal model. Consistent with the results of this study, the increase in STAT3 phosphorylation at 2 and 6 hours after a bout of resistance training has been observed (Begue et al., 2013). The results of Taneri et al. (2008) also indicate that STAT3 phosphorylation increases in the skeletal muscles of elderly subjects (23 times) and in young subjects (5 times) after intense resistance training. Significant increase of STAT3 were associated with the induction of STAT3 target genes including; Interleukin-6 (IL-6), transcription factor jun-B (JUNB),

c-MYC gene and inhibitor of cytokine signaling (SOCS) 3 mRNA after exercise (Trenerry et al., 2008). Some research has also shown that endurance training and pre-training with interval training is associated with increased phosphorylation of the signaling pathway in the hypothalamus and heart. In this regard, Zhao et al. (2011) stated that 9 weeks of endurance training protocol significantly increased STAT3 in the hypothalamus of rats (Zhao et al., 2011).

Sun and Mao (2018) also showed that preconditioning with intermittent exercise significantly reduced ischemic injury caused by resistance exercise, which was associated with a decrease in the level of myocardial infarction and an increase in STAT3 phosphorylation in male rats (Sun & Mao, 2018). IL-22 signaling has been shown to utilize Janus kinase 1 (Jak1) and tyrosine kinase-2 (Tyk2) to propagate downstream phosphorylation signals involving STAT3 and MAPK signaling pathways (Ikeuchi et al., 2005; Lejeune et al., 2002). Ekiuchi et al. investigated that IL-22 can increase extracellular signal-regulated kinase 1/2 (ERK) and phosphorylated p38. It has been found that rhIL-22 can activate the STAT3 pathway by increasing the expression of phosphorylated STAT3. Accordingly, STAT3 phosphorylation is an essential pathway in mediating the effects of IL-22 (Yang & Zheng, 2014). However, contrary to the findings of the present research, the results of Pathamaparapanan et al. (2016) did not show a change in STAT3 in the muscles of normal and diabetic rats (Pattamaprapanont et al., 2016). Tennery et al. (2011) also reported that 12 weeks of intense resistance training transiently increased STAT3 phosphorylation in young men, suggesting that these factors are not modulated by training and may only be a major component of adaptive responses to intense exercise (Trenerry et al., 2011).

Probably, the inconsistency of the results of the present research with the above findings can be attributed to the type of tissue examined, which was examined in this research, liver tissue;on the other hand, the type of subjects, the intensity of the exercise and the duration of the exercise protocol are also different. According to the results of this study, it seems that high-intensity interval training by regulating the expression of IL-22 and STAT3 genes in the fatty liver model can bring benefits for these subjects, but according to the limited studies in this ground, the need for more research is necessary. Among the limitations of the current research, we can mention the lack of measurement of pro-inflammatory and anti-inflammatory indicators to better explain the possible mechanisms of IL-22 changes in response to intense intermittent exercise. According to the research conducted in this field, it seems that the effect of exercise on the levels of adipokines in fatty liver disease requires more studies to determine the effective mechanisms on the changes of adipokines, and on the other hand, it is possible that IL-22 under the effect of individual variability in cardiometabolic responses to exercise interventions or dietary intake.Therefore, it is suggested to evaluate the effect of intense intermittent exercise on IL-22 changes in human subjects with fatty liver disease with diet control in future studies.

Conclusion

In sum, the findings of the present research show that intense interval exercise led to a significant decrease in the expression of the IL-22 gene and a significant increase in the expression of the STAT3 gene in the steatosis animal model. Therefore, according to the results, it seems that intense intermittent exercises can help improve the condition of liver steatosis.

What is already known on this subject?

Inflammation is a major component of almost all acute and chronic liver disorders, including non-alcoholic fatty liver disease.

What this study adds?

Intense interval exercise led to a significant decrease in the expression of the IL-22 gene and a significant increase in the expression of the STAT3 gene in the steatosis animal model.

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Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving animal participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Declaration of Helsinki.

Informed consent done.

Author contributions

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

References

Alvarenga-Filho, H., Sacramento, P. M., Ferreira, T. B., Hygino, J., Abreu, J. E. C., Carvalho, S. R., . . . Bento, C. A. J. J. o. n. (2016). Combined exercise training reduces fatigue and modulates the cytokine profile of T-cells from multiple sclerosis patients in response to neuromediators. 293, 91-99. doi: https://doi.org/10.1016/j.jneuroim.2016.02.014

Beavers, K. M., & Nicklas, B. J. J. F. i. b. (2011). Effects of lifestyle interventions on inflammatory markers in the metabolic syndrome. 3, 168. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3665333/

Begue, G., Douillard, A., Galbes, O., Rossano, B., Vernus, B., Candau, R., & Py, G. J. P. o. (2013). Early activation of rat skeletal muscle IL-6/STAT1/STAT3 dependent gene expression in resistance exercise linked to hypertrophy. 8(2), e57141. doi: https://doi.org/10.1371/journal.pone.0057141

Carmo, R. F., Cavalcanti, M. S., & Moura, P. J. C. (2017). Role of Interleukin-22 in chronic liver injury. 98, 107-114. doi: https://doi.org/10.1016/j.cyto.2016.08.023

Dalmas, E., Venteclef, N., Caer, C., Poitou, C., Cremer, I., Aron-Wisnewsky, J., . . . Clément, K. J. D. (2014). T cell–derived IL-22 amplifies IL-1β–driven inflammation in human adipose tissue: Relevance to obesity and type 2 diabetes. 63(6), 1966-1977. doi: https://doi.org/10.2337/db13-1511

Dudakov, J. A., Hanash, A. M., & van den Brink, M. R. J. A. r. o. i. (2015). Interleukin-22: immunobiology and pathology. 33, 747-785.

Ferrante, R. J., Andreassen, O. A., Jenkins, B. G., Dedeoglu, A., Kuemmerle, S., Kubilus, J. K., . . . Beal, M. F. J. J. o. N. (2000). Neuroprotective effects of creatine in a transgenic mouse model of Huntington's disease. 20(12), 4389-4397. doi: https://doi.org/10.1523/JNEUROSCI.20-12-04389.2000

Gibala, M. J., Little, J. P., MacDonald, M. J., & Hawley, J. A. J. T. J. o. p. (2012). Physiological adaptations to low-volume, high-intensity interval training in health and disease. 590(5), 1077-1084. doi: https://doi.org/10.1113/jphysiol.2011.224725

Ikeuchi, H., Kuroiwa, T., Hiramatsu, N., Kaneko, Y., Hiromura, K., Ueki, K., . . . Rheumatism. (2005). Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. 52(4), 1037-1046. doi: https://doi.org/10.1002/art.20965

Kalaki-Jouybari, F., Shanaki, M., Delfan, M., Gorgani-Firouzjae, S., Khakdan, S. J. A. o. p., & biochemistry. (2020). High-intensity interval training (HIIT) alleviated NAFLD feature via miR-122 induction in liver of high-fat high-fructose diet induced diabetic rats. 126(3), 242-249. doi: https://doi.org/10.1080/13813455.2018.1510968

Keating, S. E., George, J., Johnson, N. A. J. E. r. o. g., & hepatology. (2015). The benefits of exercise for patients with non-alcoholic fatty liver disease. 9(10), 1247-1250. doi: https://doi.org/10.1586/17474124.2015.1075392

Ki, S. H., Park, O., Zheng, M., Morales-Ibanez, O., Kolls, J. K., Bataller, R., & Gao, B. J. H. (2010). Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. 52(4), 1291-1300. doi: https://doi.org/10.1002/hep.23837

Lejeune, D., Dumoutier, L., Constantinescu, S., Kruijer, W., Schuringa, J. J., & Renauld, J.-C. J. J. o. B. C. (2002). Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line: pathways that are shared with and distinct from IL-10. 277(37), 33676-33682. doi: https://doi.org/10.1074/jbc.M204204200

Mo, R., Lai, R., Lu, J., Zhuang, Y., Zhou, T., Jiang, S., . . . Liu, Y. J. T. (2018). Enhanced autophagy contributes to protective effects of IL-22 against acetaminophen-induced liver injury. 8(15), 4170. doi: https://doi.org/10.7150/thno.25798

Niederreiter, L., & Tilg, H. J. L. R. (2018). Cytokines and fatty liver diseases. 2(1), 14-20. doi: https://doi.org/10.1016/j.livres.2018.03.003

Pasarica, M., Sereda, O. R., Redman, L. M., Albarado, D. C., Hymel, D. T., Roan, L. E., . . . Smith, S. R. J. D. (2009). Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. 58(3), 718-725. doi: https://doi.org/10.2337/db08-1098

Pattamaprapanont, P., Muanprasat, C., Soodvilai, S., Srimaroeng, C., & Chatsudthipong, V. J. T. r. o. d. s. R. (2016). Effect of exercise training on signaling of interleukin-6 in skeletal muscles of type 2 diabetic rats. 13(2-3), 197. doi: https://doi.org/10.1900/RDS.2016.13.197

Ramos, J. S., Dalleck, L. C., Stennett, R. C., Mielke, G. I., Keating, S. E., Murray, L., . . . Therapy. (2020). Effect of Different Volumes of Interval Training and Continuous Exercise on Interleukin-22 in Adults with Metabolic Syndrome: A Randomized Trial. 13, 2443. doi: https://doi.org/10.2147/DMSO.S251567

Ramos, J. S., Dalleck, L. C., Tjonna, A. E., Beetham, K. S., & Coombes, J. S. J. S. m. (2015). The impact of high-intensity interval training versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. 45, 679-692. doi: https://doi.org/10.1007/s40279-015-0321-z

Rangel, M., Renno, A. S., Oliveira-Junior, M. C., de Moraes, G., Mahler, J. d. A. C., Soares, C. R., . . . Belvisi, M. G. (2017). Involvement of STAT-3 in the Beneficial Effects of Aerobic Exercise in a Model of Smoke-Induced COPD. In: Eur Respiratory Soc.doi: https://doi.org/10.1183/1393003.congress-2017.PA3728

Shabana, M., Ibrahim, H. M., Khadre, S. E., Elemam, M. G. J. T. J. o. B., & Zoology, A. (2012). Influence of rifampicin and tetracycline administration on some biochemical and histological parameters in albino rats. 65(5), 299-308. doi: https://doi.org/10.1016/j.jobaz.2012.10.009

Slentz, C. A., Tanner, C. J., Bateman, L. A., Durheim, M. T., Huffman, K. M., Houmard, J. A., & Kraus, W. E. J. D. c. (2009). Effects of exercise

training intensity on pancreatic β-cell function. 32(10), 1807-1811. doi: https://doi.org/10.2337/dc09-0032

Steckling, F., Farinha, J., Santos, D., Bresciani, G., Mortari, J., Stefanello, S., . . . diabetes. (2016). High intensity interval training reduces the levels of serum inflammatory cytokine on women with metabolic syndrome. 124(10), 597-601.

Sun, X., & Mao, J. J. E. R. M. P. S. (2018). Role of Janus kinase 2/signal transducer and activator of transcription 3 signaling pathway in cardioprotection of exercise preconditioning. 22(15), 4975-4986. URL: https://www.europeanreview.org/wp/wp-content/uploads/4975-4986.pdf

Tjønna, A. E., Lee, S. J., Rognmo, Ø., Stølen, T. O., Bye, A., Haram, P. M., . . . Slørdahl, S. A. J. C. (2008). Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study. 118(4), 346-354. doi: https://doi.org/10.1161/CIRCULATIONAHA.108.772822

Trenerry, M. K., Carey, K. A., Ward, A. C., Farnfield, M. M., & Cameron-Smith, D. J. R. r. (2008). Exercise-induced activation of STAT3 signaling is increased with age. 11(4), 717-724.

Trenerry, M. K., Della Gatta, P. A., Larsen, A. E., Garnham, A. P., Cameron‐Smith, D. J. M., & nerve. (2011). Impact of resistance exercise training on interleukin‐6 and JAK/STAT in young men. 43(3), 385-392. doi: https://doi.org/10.1002/mus.21875

Woo, G. A., & O'Brien, C. J. C. i. l. d. (2012). Long-term management of alcoholic liver disease. 16(4), 763-781.

Wu, Y., Min, J., Ge, C., Shu, J., Tian, D., Yuan, Y., & Zhou, D. J. I. J. o. B. S. (2020). Interleukin 22 in liver injury, inflammation and cancer. 16(13), 2405. doi: https://doi.org/10.7150/ijbs.38925

Yang, L., Zhang, Y., Wang, L., Fan, F., Zhu, L., Li, Z., . . . Huang, Z. J. J. o. h. (2010). Amelioration of high fat diet induced liver lipogenesis and hepatic steatosis by interleukin-22. 53(2), 339-347. doi: https://doi.org/10.1016/j.jhep.2010.03.004

Yang, X., & Zheng, S. G. J. A. r. (2014). Interleukin-22: a likely target for treatment of autoimmune diseases. 13(6), 615-620. doi: https://doi.org/10.1016/j.autrev.2013.11.008

Zhao, J., Qi, Y.-F., & Yu, Y.-R. J. A. o. h. (2021). STAT3: A key regulator in liver fibrosis. 21, 100224.

Zhao, J., Tian, Y., Xu, J., Liu, D., Wang, X., Zhao, B. J. L. i. h., & disease. (2011). Endurance exercise is a leptin signaling mimetic in hypothalamus of Wistar rats. 10(1), 1-7. doi: https://doi.org/10.1186/1476-511X-10-225

Ziesché, E., Bachmann, M., Kleinert, H., Pfeilschifter, J., & Mühl, H. J. J. o. B. C. (2007). The interleukin-22/STAT3 pathway potentiates expression of inducible nitric-oxide synthase in human colon carcinoma cells. 282(22), 16006-16015. doi: https://doi.org/10.1074/jbc.M611040200