

The effect of irisin on thyroid function and some immunological factor in Iraqi women practicing sport

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ABSTRACT

Irisin is a new myokine generated in mice and humans by proteolytic cleavage of fibronectin, and it may also be released in tiny amounts by adipose tissue and the liver. Irisin acts as an adipokine and myokine hormone that is released after exercise. The present study aimed to know the effect of irisin values on the levels of white blood cells and their subsets, Thyroid hormones (T3&T4) and Thyroid stimulating hormone (TSH), immunoglobulin (IgG and IgM). About 100 volunteers are Iraqi women aged (18-38) years old, the 100 volunteers are classified into two groups. The first group is the women practicing sports, whose training lasts between 3 months and 1 year or more, which includes 80 volunteers, and the second group is the control group, those who are not playing any type of sport which includes 20 volunteers. The present study showed the level of irisin had a highly significant increase in women practicing sports more than control ($p \leq 0.01$) by using the ELISA kit. We found a highly significant increase ($P \leq 0.01$) in WBC (white blood cell) in women practicing sports compared to control women. When going for a thyroid function test, the present study found a significant increase ($P < 0.05$) in T3 and T4 but a significant decrease ($P < 0.05$) in TSH in women practicing sports in comparison to control. In immunity levels, the IgG and the IgM were found to have a highly significant increase ($P < 0.001$) in women practicing sports in comparison to control. On the other hand, we found no significant difference ($P \geq 0.05$) in lymphocyte, eosinophil and basophil in women practicing sports, but in monocyte we found a significant decrease ($P < 0.05$) in neutrophils we found a significant increase ($P < 0.05$) in women practicing sports compared to control.



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1. Introduction

Skeletal muscle is progressively being identified as an endocrine organ that secretes different signaling molecules known as myokines. These myokines regulate various physiological processes. Lately, [1] showed that a new myokine called irisin exists, This name is a derivative of the Greek goddess Iris [2]. As has been said previously, irisin, recently discovered, is a putative myokine that is induced by exercise and it is made

by cleavage of the FNDC5, which refers to the fibronectin domain containing protein 5 receptor. As known, irisin is a myokine that is released after exercise and that is connected with increased energy expenditure because of its capacity to catalyze the browning of white adipose tissue (WAT). It's still unknown which type of exercise, intensity, or duration, if any, is best for raising circulating blood irisin levels in the general population [2]. Irisin is a novel hormone released by myocytes that has been suggested to mediate the beneficial influences of exercise on metabolism [3]. Irisin could be a helpful biomarker in the differential diagnosis of acute abdominal aches in children, and it could be included in laboratory assays, because the discovery of elevated blood irisin levels in the vermiform appendix, a tubular organ, after infection and perforation suggests that irisin could be employed as a diagnostic marker for these disorders [4].

Studies examining circulating irisin in thyroid diseases, especially overt hypo and hyperthyroidism and subclinical hyperthyroidism are extremely rare [5]. Comparison to the obese patients in the healthy control group, the hypothyroidism group's obese patients' serum irisin levels were found to be greater. Additionally, it was shown that the severity of hypothyroidism increased in conjunction with the serum irisin level (with increased TSH and decreased fT4) [6]. The studies by [7] describe the relationship between irisin and the thyroid gland and suggest that irisin is lower in hypothyroid while it is higher in hyperthyroid and suggest that there is a negative correlation between irisin and TSH. In patients with newly diagnosed autoimmune overt hypothyroidism caused by Hashimoto's thyroiditis, the same research showed a rise in serum irisin levels once euthyroidism was attained by levothyroxine medication [8]. The adipogenesis of WAT and BAT is influenced by TSH, thyroid hormones, and irisin, which also causes the browning of WAT [5].

During exercise, there is an inflammatory state and a significant generation of free radicals, both of which affect iron metabolism and have a direct influence on iron reduction due to its affinity for H₂O₂. Furthermore, skeletal muscle is a highly regenerative tissue, but its repair capacity is limited, and inflammatory signaling aids in muscle repair [9]. The rise in IgG levels during rest in volleyball players was caused by (post)stress inflammatory processes that led to an increase in the amount of circulating lymphocytes concentration both after a single workout and throughout the competition season. In contrast, a single exercise test significantly increased IgG levels before and after training [10]. There is a link between irisin and IgM, since irisin serum levels were shown to be greater in persons with IgM when compared to serum protein levels in healthy individuals [11]. [12] discovered that boosting IgG levels in people with immunoassay illness increases exercise ability. Strong training for 5 weeks in female rats has an effect on the acquired immune system, increasing blood IgG concentration and in vitro IgM production [13].

The work aims to study the physiological and immunological effect of irisin hormone in women practicing sports compared to control women to study the effect of exercise on the level of the irisin hormone and to study the physiological effect of irisin on thyroid gland and the levels of some immunological factor.

2. Material and methods

About 100 volunteers are Iraqi women aged (18-38) years old from College of Physical Education in Al-Mustansiriyah University and from different gyms in various areas of Bagdad during the period extended between November/2021 to February/2022. However, the 100 volunteers are classified into two groups. The first group is the Women practicing sports group, whose training lasts between 3 months and 1 year or more, which includes 80 volunteers, and the second group is the control group, those who are not playing any type of sport which includes 20 volunteers, the volunteers had a negative history of any medical condition or surgery, and they were all fasting before blood was collected to determine a variety of special testing. About 5ml from venipuncture by using sterilized disposable syringes from both women practicing sports and the control group. Then 2 ml of the blood samples were transferred into EDTA-containing tubes

and mixed very gently about three times, and the remaining 3 ml of blood samples were transferred into gel and clot activator tubes, and both the tubes were put into an icebox containing a refrigerated ice gel pack. The EDTA tubes are then placed in a roller mixer for about 15 minutes in the lab to determine a some parameters of complete blood count (CBC) like White blood cells (WBCs), Lymphocyte, Monocyte, Neutrophil, Eosinophil, Basophil, platelets and platelets distribution width (PDW)and fully automatic quantitative MS4s®CBC Automated Device is used for the determination of these parametersand the gel clot activator tubes are placed in a centrifuge to separate serum at 3000 rpm for 10 minutes. All serum from samples is transferred into Eppendorf tubes, labeled with the name of the person and date of collection, and stored at -20 °C until used. The serum that was separated from the gel tube is used to determine the rest of the analysis. Serum irisin measured by using human irisin ELISA Kit (enzyme-linked immunosorbent assay) (SunLong Biotech Co.,LTD, China), to measured thyroid function test using cobas e411fully automated device (Roche Diagnostics, Hitachi, USA) and to measured immunoglobulin assay using cobas c501fully automated device(Roche Diagnostics, Hitachi, USA).To determine the influence of different components in research parameters, the Statistical Analysis System SAS (2012) application was utilized. In this investigation, the T-test was employed to compare means that were statistically significant [14].

3. Result

The current study found that the level of irisin hormone highly significantly increased ($p \leq 0.01$) in women practicing sports group (5.78 ± 0.19) compared with control group (3.74 ± 0.07) as shown in Table (3-1).

Table (3-1): The levels of Irisin hormones between women practicing sports and control groups.

Parameters	Women practicing sports	Control	T-test	P-value
Irisin (ng/ml)	5.78 ± 0.19	3.74 ± 0.07	9.84	0.001**

The values represent mean \pm SE.

** significant is under the (0.01 level).

The result of this study demonstrated highly statistically significant increase ($P \leq 0.01$) in WBC level between the women practicing sports group (8.19 ± 0.29) and control group (6.74 ± 0.36). On the other hand, there is no significant deferens in lymphocyte, eosinophil and basophil between women practicing sports group (29.71 ± 0.90), (2.42 ± 0.25) (0.418 ± 0.04) and control group (32.03 ± 1.69), (3.06 ± 0.35), (0.481 ± 0.04) respectively. On the other side, there is a significant decrease between women practicing sports group (4.26 ± 0.14) and control group (4.92 ± 0.24) in monocyte at ($P < 0.05$) and also at the same level ($P < 0.05$) there is a significant decrease in neutrophil level between women practicing sports group (63.33 ± 0.95) and control group (59.33 ± 1.77) as showed in Table (3-2).

Table (3-2): Comparison between women practicing sports and control groups in differential of WBC.

Parameters	Women practicing sports	Control	T-test	P-value
WBC(m/mm^3)	8.19 ± 0.29	6.74 ± 0.36	0.971	0.001**
Lymphocyte(%)	29.71 ± 0.90	32.03 ± 1.69	3.497	0.224
Monocyte(%)	4.26 ± 0.14	4.92 ± 0.24	0.530	0.01*
Neutrophils(%)	63.33 ± 0.95	59.33 ± 1.77	3.679	0.04*
Eosinophil(%)	2.42 ± 0.25	3.06 ± 0.35	0.885	0.149
Basophil(%)	0.418 ± 0.04	0.481 ± 0.04	0.136	0.394

The values represent mean \pm SE.

* significant is under the (0.05 level).

** significant is under the (0.01 level).

The represent result indicate a significant increased ($P=0.05$) in T3 and T4 between women practicing sports group (2.89 ± 0.07), (108.04 ± 2.71) and control group (1.64 ± 0.08), (76.02 ± 1.29) respectively. While the levels of TSH is significant decreased ($P=0.05$) between women practicing sports group (0.531 ± 0.04) and in control group (1.78 ± 0.18) as shown in Table (3-3).

Table (3-3): Comparison between women practicing sports and control groups in T3,T4 and TSH hormones level.

Parameters	Women practicing sports	Control	T-test	P-value
T3(nmol/L)	2.89 ± 0.07	1.64 ± 0.08	0.224	0.05*
T4(nmol/L)	108.04 ± 2.71	76.02 ± 1.29	6.14	0.05*
TSH(μ IU/mL)	0.531 ± 0.04	1.78 ± 0.18	0.397	0.05*

The values represent mean \pm SE.

* significant is under the (0.05 level).

Table (3-4) shown that levels of IgG and IgM were high significantly increased ($P=0.01$) in women practicing sports group (1380.27 ± 47.14) (242.40 ± 22.90) compared to control group (827.46 ± 49.16) (77.20 ± 3.93) respectively.

Table (3-4): Comparison between women practicing sports and control groups in IgG and IgM levels.

Parameters	Women practicing sports	Control	T-test	P-value
IgG(mg/dL)	1380.27 ± 47.14	827.46 ± 49.16	139.52	0.001**
IgM(mg/dL)	242.40 ± 22.90	77.20 ± 3.93	47.606	0.001**

The values represent mean \pm SE.

** significant is under the (0.001 level).

4. Discussion

From these results, noticed that the women practicing sports group had highest value of irisin. These back to reasons, the increase of the value in women practicing sports, due to release after exercise and that is connected with increased energy expenditure because of its capacity to catalyze the browning of white adipose tissue (WAT) to brown adipose tissue (BAT) [15]. Irisin increases white-to-brown fat conversion, it is thought to work as a myokine, binding to and activating an undiscovered receptor [16]. [1] works both in culture and *in vivo*, showed that a novel polypeptide hormone called irisin, that is produced from muscle into the circulation and promotes thermogenic action in adipose tissues and it is controlled by PGC1- α . Irisin levels in the blood rose in response to acute exercise but dropped following medically induced weight reduction [3]. [17] add a new update to Boström research that irisin is not only a myokine but also an adipokine. Another study indicated that irisin secreted after one year of training are increased significantly [18]. [19] found that during moderate intensity continuous aerobic exercise at afternoon, the irisin level increased significantly in female sports group. In animals, in 2021 provides evidence that irisin induced in mice by exercise [20]. The current study agrees with many studies that indicated a significant increase in the level of the irisin hormone after exercise [21- 23]. Some investigation works on 2 type of group, the first one indoor group found that after first training the serum concentration of irisin increased significantly, then after 18 week the irisin concentration decreased significantly, the second group called outdoor group, in this group found that after first training the serum concentration of irisin increased significantly but after 18 week the concentration of irisin remain constant [24].

On the other side, there is investigation that have opposite opinion and do not agree with the result. Other

found no significant changes in serum irisin levels were detected in the exercised mice [25]. Another study also found highly significant decrease in irisin value in group with chronic exercise compared to control group in adult [26].

Our findings show that the reaction to exercise differed considerably between exercise and non-exercise women. This high rise in plasma irisin explains a portion of the metabolic response to exercise in female athletes.

Another study found that blood samples from 14 male and 23 female athletes who exercised regularly for 12 weeks did not demonstrate a significant change in WBC counts. [27]. Another study found that intense training for 5 weeks has an influence on the acquired immune system in female rats. The effect on the distribution of lymphocytes between compartments, encompassing both main and secondary lymphoid tissues [13]. In obese people, there is study demonstrated that resting irisin levels in obese people increased in response to WBC [28]. The effectiveness of WBC in treating the symptoms of all types of inflammatory disorders that could affect athletes is demonstrated by [29]. These findings, along with evidence that WBC resembles exercise, at least in terms of its ability to stimulate pulsatile production of the myokine irisin.

[30] revealed that the adhesive interactions of lymphocytes with irisin, as well as the investigation of irisin's biological involvement in the control of leukocyte migration in inflammation. As indicated by one of the previous studies that Lymphocyte, Neutrophils and Eosinophil increased with the increase of irisin, while monocyte and basophil did not effected by increase in irisin value [31]. [32] discovered that, in addition to dehydration, elevated catecholamine levels during exercise cause WBC recruitment, with a connection between the rise in WBC and heart rate following a brief period of hard cycling. [33] found that in volleyball players, the number of circulating lymphocytes rose after accumulating exercise stress over the competition season as well as after a single workout, but body weight reduced insignificantly and physical ability improved. These alterations were accompanied by an increase in the number of leukocytes and their subsets (leukocyte subset count) as well as the IgG level at rest. Another study showed that Leukocytes and neutrophils significantly increase, while Lymphocytes and Monocytes found no significant effect, also Eosinophils and Basophils show significant decrease between training group and control group after training period [9]. In women, same research found that when value of irisin increased significantly, the value of leukocytes and there subsets like (Neutrophils, Lymphocytes and Eosinophils) also increased significantly [34].

[13] established that the time and kind of exercise affect the blood lymphocyte response following the lengthier training, the proportions of blood lymphocyte subsets altered during the exhaustion exercise, indicating a fast lymphocytosis after exercise. The biological functions of irisin in controlling leukocyte migration during inflammation are still being investigated. As increasing evidence indicates to irisin's important involvement in metabolic control, future research into the biology of irisin may show that irisin is at the heart of metabolic regulation of leukocyte migration [30]. [35] found there is other positive correlation was found between serum irisin and WBC count. Exercise in the morning and the evening had different effects on respondents' hematological levels in terms of an increase or reduction, a significance increase in the value of leukocytes; WBC and neutrophil, and was a significance decrease lymphocyte, between the morning and evening exercise [36]. Since irisin's discovery, a lot of focus has been placed on its possible therapeutic use in the treatment of a number of endocrine and metabolic disorders. It may be hypothesized that irisin and thyroid function are related given that both irisin and thyroid hormones are linked to metabolism, muscle physiology, and body energy consumption as well as the fact that serum irisin concentrations differ based on TSH and T4 in thyroid dysfunction [37]. Circulating irisin levels were found

to be lower in hypothyroid people, particularly those with clinical hypothyroidism and autoimmune thyroid disease. The relationship between irisin, thyroid hormones, and antibodies suggested that they may interact to control energy metabolism [38], [39]. Another study demonstrated that had either hypothyroidism or hyperthyroidism that irisin is lower in hypothyroidism rather than in hyperthyroidism, but this study did not have a control group [40]. [41] showed that hyperthyroid patients had higher irisin level than hypothyroid patient with a high significant difference. Because irisin levels were significantly lower in hypothyroid patients than in healthy participants in Chinese patients, irisin hormone was positively associated with T3 and T4 levels and negatively associated with TSH levels [42]. Another study by [43] found that swimming exercise have significant increase in both serum T4 and T3 and a significant decrease in TSH compared to hypothyroid rats, and these results agreed with the results of our study.

One study indicates a positive relationship between thyroid hormones and irisin, [44] found that the mean irisin level was higher in the hyperthyroid group than the hypothyroid and control group and also found a significant correlation between the TSH, T3, T4 levels and irisin levels and found that as TSH levels increased, irisin levels decreased and as T3, T4 levels increased, irisin levels increased. On other hand, [6] discovered that serum irisin levels were greater in the hypothyroidism group in comparison to the control group, and that serum irisin concentrations increased in association with the severity of hypothyroidism, as measured by elevated TSH and decreased T4. While another study, discovered that in the whole population, irisin level had a positive correlation with TSH but a negative correlation with T4. Patients with long lasting hypothyroidism have lower irisin levels than those with short term disorder [45].

In Iraqi patients [46] observed with the hyperthyroid and the euthyroid control groups have lower serum irisin value than hypothyroidism means irisin value correlated positively with TSH and negatively with T3 and T4. Another research found that low blood irisin levels rose considerably after therapy to euthyroid condition in overt hypothyroid individuals with Hashimoto thyroiditis [8]. The physiological effects of irisin on the hypothalamic-pituitary thyroid axis (HTP) and energy metabolism are demonstrated in this study.

In general, exercising at a moderate to vigorous intensity for 60 minutes or less is optimal for the immune-boosting benefits of exercise because improves the anti-pathogen activity of tissue macrophages in parallel with an enhanced recirculation of immunoglobulins and also effect to enhance immune defense activity and metabolic health, but high intensity training have opposite effect on the body [47]. The intensity of exercise in different oxygen settings has varying impacts on different immune functions, and has considerable effects on IgG, IgM, and WBC, which may be used to assess effective immunological indices [48]. [12] found increasing in IgG in patients with immunoassay disease improves exercise capacity in those patients. The acquired immune system is affected by hard training for 5 weeks in female rats, increasing serum IgG concentration and in vitro IgM production [13]. Another study demonstrated that there is highly significant increase in IgG in adult male rats after 1 day of exercise [49]. The acute exercise in women led to increased serum IgG, and IgM levels [50], [51]. [33] revealed that greater lung circulation during exercise may be connected with an increase in the respiratory system's resistance to infections, which may be indicated by rising IgG levels. Exercise induced liver β 2-microglobulin expression is related to lower IgG clearance in the blood [52]. Previous study suggest that IgG concentration decrease in exercised rats, while IgM decreased in both sedentary and runner rats [53]. [54] show no statistical different in IgG between anaerobic and aerobic groups before and after 14 weeks training, in contrast to IgM which decreased. In men a study found that high training decreased IgG concentration [55].

On other hand; same researcher didn't found any significant difference or in IgG and/or IgM concentration

[56], [57]. The cause of immunoglobulin highly significant in women practicing sports compared to control, is effect to enhance immune defense activity and metabolic health and also, exercise raises body temperature. Regular exercise training has an anti-inflammatory effect that is mediated by numerous pathways [47].

5. Conclusion

The result of this study involved exercising has a significant role in increasing the level of irisin in group of women practicing sports compared to the control group. WBC, Neutrophils, and Monocyte increased significantly in women practicing sports compared to control group. The results indicate a significant increase in the levels of thyroid hormones T3 and T4, with a significant decrease in the level of TSH in women practicing sports in comparison to the control group. This indicates that irisin has a role in stimulation and the activity of thyroid hormones. IgG and IgM increased significantly in women practicing sports in comparison to the control group. This increase may be because the role of irisin in activating the immune system and local infections.

A bigger sample size and samples from other Iraqi locations will be used in future research on irisin. examination of the irisin hormone's impact on the female reproductive system. examination of the irisin hormone's impact on the male reproductive system. A comparison of the irisin hormone levels in males and females both before and after exercise. investigating how the irisin hormone affects certain metabolic diseases including obesity and insulin resistance. examining the impact of irisin hormone in physically active polycystic ovary syndrome patients.

6. References

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