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MYOKINE IRISIN AND ITS METABOLIC ROLE ON *TRICHINELLA SPIRALIS* IN EXPERIMENTALLY INFECTED MURINE MODEL

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Abstract

Trichinella spiralis infects striated muscle cells and causes one of the most important parasitic diseases. The rapidly growing intramuscular larvae depend on insulin signaling pathways to supply their needs of glucose and glycogen. Irisin is a myokine secreted by skeletal muscles during exercise to increase energy expenditure by stimulating glucose uptake and accumulation of glycogen in muscle cells. This study investigated the potential role of irisin during experimental trichinosis. Thirty albino mice were infected orally with *T. spiralis* (200larva/mouse) and five healthy mice were assigned as normal control. On the 7th, 14th, 21st, 28th, 35th, &48th day post-infection, mice were evaluated regarding the changes in body weight, blood glucose level, serum insulin, histopathological changes, glycogen storage, and immunohistochemical expression of irisin. The results revealed that during the early stage, from the 7th to 28th dpi there were gradual insignificant decreases in mice body weight. Blood glucose levels showed significant decreases and the lowest was on the 21st and 28th dpi (83.6±3.05 and 95.6±5.08, respectively). Also, during this phase, there were significant increases in serum insulin and the highest was on the 21st dpi (8.7±0.34). These changes correlated with the development and growth of nurse cells parallel with increased glycogen accumulation and irisin expression in muscle bundles. During the late stage, there were significant decreases in body weight, significant increases in blood glucose levels, and significant decreases in serum insulin. The histopathological examination revealed intense cellular inflammatory infiltrate associated with glycogen depletion. Strong irisin expressions were observed in the inflammatory infiltrates, nerve bundles, and the adjacent adipose tissues while in muscle bundles it decreased. In conclusion, the increased irisin expression in muscle bundles is suggested to increase insulin secretion and responsiveness of *T. spiralis*-infected muscles to facilitate glucose transport and glycogen accumulation during larval growth. Whereas, during the late stage, irisin affects fat metabolism and might contribute to loss of body weight. The increased irisin expression in the inflammatory infiltrates may have protective and anti-inflammatory roles. However, further studies should be conducted to discover in-depth role of irisin and host-endocrine interplay during *T. spiralis* infection.

Keywords: *T. spiralis*, irisin, insulin, blood glucose, glycogen, immunohistochemistry, PAS

Introduction

Trichinosis is one of the most important food-borne parasitic zoonotic diseases caused by *Trichinella spiralis*. This parasitic nematode infects striated muscle cells of a diversity of mammalian hosts, including humans (Murrell *et al*, 2000). The disease is of global distribution, including Europe, Southeast Asia, North and South America, and North Africa (Yan *et al*, 2021).

Trichinella spiralis infection in humans is strongly related to eating pork meat. However, wild carnivorous animals can harbor and continue infections of *T. spiralis* and other species in the sylvatic cycle. Therefore, walrus, bears, cougars, and wild boars can also be sources of infection (Morsy *et al*, 2022). Herbivorous animals such as cattle are not naturally infected, but beef meat may become contaminated, either intentionally by illegal mixing with meat of unknown origin or incidentally during meat processing by using a grinder previously used for mincing pork (Huong et al, 2017; Morsy *et al*, 2022). These reasons may explain how the seroprevalence of trichinosis was 10% in a

study done in Egypt, although most Egyptians abstain from eating pork or its products due to the religious laws (Mohammed *et al*, 2022).

After consumption of undercooked infected meat of wild animals and pigs, the first-stage larvae of *T. spiralis* inhabit the intestinal epithelium where they develop into adults that copulate within 3 to 7 days and produce fully mature newborn larvae (Yu *et al*, 3013). These newborn larvae leave the small intestine through the lymphatics and vascular system. During their migration, larvae can penetrate different cell types of highly vascularized tissues. However, only striated muscle cells support the growth, development, and survival of *T. spiralis* larvae (Piaggi *et al*, 2021).

Once the newborn larvae have established themselves inside a muscle cell, they grow and transform muscle cells into a unique cell type, called 'nurse cells' (Piaggi *et al*, 2021). This larval growth phase takes about 20-30 days (Despommier, 1983) where the muscle-enclosed larvae can survive for many years (Sacchi *et al*, 2001), and transformation of muscle cells into nurse cells occurs after biochemical and morphological alterations, which help nurse cells to perform their functions of protection, supplying nutrients to larvae and meeting their own metabolic and nutritional requirements (Montgomery *et al*, 2003).

The skeletal muscle is an important organ of the body and recent evidence showed that skeletal muscle is not only an organ for locomotion, but it secrets several physiological factors known as myokines (Pedersen, 2011). Myokines communicate with other organs, such as adipose tissue, the liver, and brain, in an autocrine, paracrine, or endocrine manner (Pedersen *et al*, 2007). They influence a variety of physiological functions, such as myogenesis, osteogenesis, fat oxidation, endothelial function, and glucose hemostasis. The identified myokines include several cytokines and molecules, such as IL-6, IL-7, IL-15, fibroblast growth factor-21, insulin-like 6, musclin, and the newly discovered irisin (Lee *et al*, 2015).

Bostrom *et al*. (2012) discovered Irisin by as a previously uncharacterized hormone derived from the transmembrane protein fibronectin type III domain-containing protein-5 (FNDC5). Irisin is widely expressed in several tissues, and it may be defined as a myokine or an adipokine released during exercise (Roca-Rivada *et al*, 2013). In response to exercise, it works to increase energy expenditure by promoting the browning of white adipose tissues resulting in thermogenesis (Zhang *et al*, 2016; Martinez Munoz *et al*, 2018). Through irisin, skeletal muscles communicate with the pancreatic islets to regulate insulin secretion and glucose metabolism (Natalicchio *et al*, 2017). Besides, irisin stimulated glucose uptake (Lee *et al*, 2015) and accumulation of glycogen in muscle cells (Yano *et al*, 2021).

Trichinella spp. infection causes hypoglycemia in both humans and animals. This hypoglycemia was referred to high glucose consumption by the rapidly growing larvae (Wu *et al*, 2009). Insulin signaling pathway was found to play a central role in supplying the needed glucose for the rapidly growing larvae. This pathway transports glucose from blood into insulin-responsive tissues (e.g. skeletal muscles) and helps conversion of glucose into stored glycogen molecules (Youngren, 2007).

The irisin role in skeletal muscles and glucose homeostasis during *T. spiralis* infection was not yet studied. This study aimed to investigate interaction between trichinosis and irisin expression in a time-course experimental animal study. The assessment was done by measuring animal body weight, blood glucose level, and serum insulin in relation to the histopathological changes, glycogen storage, and immunohistochemical expression of irisin in skeletal muscles of experimentally infected mice with *T. spiralis*.

Materials and methods

Experimental animals and ethical approval: The present study was conducted on 35

pathogen-free Swiss albino male mice, 4-6 weeks of age, weighing on average 23-25g. and purchased from Theodore Bilharz Research Institute (TBRI), Giza. Mice were housed under controlled humidity and temperature and given standard food with water *ad libitum*.

Ethical consideration: All animal procedures were performed according to international regulations and guides. The protocol was approved by Ethical Committee, Faculty of Medicine, Menoufia University (IRB: 10/2022PARA4-3).

Parasite and infection: *Trichinella spiralis* was isolated from the diaphragms of naturally infected pigs in Cairo Abattoir. Using the standard artificial digestion method, larvae were maintained by consecutive passage via Albino rats and mice in TBRI. Briefly, on the 35th dpi, the infected animals were sacrificed, muscles were dissected, incubated in 1% pepsin and 1% HCl mixture in distilled water at 37°C and subjected to continuous stirring for 2hr. The digested muscles were sieved; larvae were collected, washed twice in sterile phosphate-buffered saline (PBS), and left to sediment. Each mouse was orally infected with 200 larvae (Dunn and Wright, 1985).

Study design: Thirty mice were infected with *T. spiralis* and assigned to six subgroups according to the scarification day. Also, additional uninfected five mice were used as normal control group (NC), and were sacrificed on day 0. The infected mice were sacrificed (5 mice for each day) on the 7th, 14th, 21st, 28th, 35th, and 48th dpi.

Sample collection: On each day of the described schedule, each mouse was weighed, and a drop of blood from tail was used to measure blood sugar level (mg/dl) by using an Accu-Chek® device (Roche Diagnostics, Switzerland). Mice were anesthetized and killed by cervical dislocation. Blood was collected by cardiac puncture and serum samples were prepared to insulin level. Muscle specimens were excised from skeletal muscles and diaphragms of the sacrificed mice, then fixed in 10% neutral buffered formaldehyde (pH 7.4) for histopathological and immunohistochemical studies.

Measurement of serum insulin: To measure serum insulin concentrations in sera samples of all groups, Invitrogen Mouse Insulin solid-phase sandwich ELISA Kit (ThermoFisher Scientific, USA) according to the manufacturer's instructions. In brief, all sera were diluted 2-fold with the diluent buffer. Of each standard and sample, 100µl were added into wells, covered and incubated for 2.5hr at room temperature (RT). After washing 4 times with the provided wash buffer, 100µl of 1×biotinylated anti-insulin antibody was added to each well and incubated with gentle shaking for an hour. The washing step was repeated to each well; 100µl of streptavidin–horseradish peroxidase (HRP) solution was added and incubated with gentle shaking for 45 min. Finally, the wash step was repeated then 100µl of a stop solution was added and incubated for 30 min in the dark. The reaction was measured by a spectrophotometer at 450 and 550nm and serum insulin was expressed as µIU/ml.

Histopathological study: The preserved muscle specimens were passed in ascending grades of ethanol, paraffin-embedded, sections of 5µm were cut, and stained with hematoxylin and eosin (Drury and Wallington, 1980).

Periodic Acid-Schiff reaction (PAS): Glycogen storage inside skeletal muscle fibers was evaluated by using periodic acid-Schiff reaction. Paraffin sections of skeletal muscles retrieved from the groups were deparaffinized and stained with PAS by using the standard technique. Briefly, sections were fixed in 3.7% formaldehyde in 90% ethanol for 1hr at 4°C. Slides were soaked in 1% periodic acid in distilled water for 5 min followed by a wash for 1 min in tap water and then for 5 min in distilled water. Thereafter, slides were put in Schiff's reagent for 15 min at RT, washed in distilled water for 5 min and then followed by 10 min in tap water (Wu *et al*, 2009).

Immunohistochemical study for irisin expression in muscle tissues: Immunohistochemical detection of irisin/FNDC5 protein in skeletal muscle specimens was done according to the manufacturer's instructions. Briefly, 4µm sections from paraffin-embedded muscle tissues were deparaffinized and incubated in H_2O_2 for 10 min to block endogenous peroxidase activity. After washing, Ultra V Block (Thermo-Fisher Scientific, USA) was applied for 10 min to block nonspecific binding. Sections were incubated in polyclonal anti-irisin IgG (Anti-FNDC5 antibody, Abcam, USA) for 16-24hr at 4°C. The antibody was diluted 1:100 with 0.1 M of PBS (pH 7.2) contained 0.25% sodium azide (Merck, Germany) and 2.5% bovine serum albumin (Sigma-Aldrich, USA). The sections were washed and incubated with biotinylated goat anti-rabbit IgG (Sigma-Aldrich, USA) followed by streptavidin-biotin peroxidase complex for 1hr at RT & washed in PBS for 30 min. Sections were immersed in AEC chromogen substrate (Dako, USA) for 10 min, washed with distilled water, counter-stained with Mayer's hematoxylin, dehydrated, cover-slipped with a mounting medium, and then examined under a microscope (Olympus, Tokyo, Japan), assessed, and photographed.

Statistical analysis: Data were collected, tabulated, and analyzed by statistical analysis using the SPSS (version 10) computer program. Data were expressed as mean and standard deviation (mean ± SD). Differences between groups were tested for significance by a one-way variance analysis (ANOVA) followed by a post Hoc test to detect significance between groups. Differences were significant when $P < 0.05$.

Results

There was a significant difference between groups regarding mice body weight. There was a gradual reduction of body weight over time after *T. spiralis* infection started from the 7th dpi and continued till the 28th dpi without significant differences. Significant reductions in mice body weight in the 35th dpi (21.6±2.2) and the 48th dpi (20.4±0.96) groups when compared to NC (24.46±1.48) were reported. Difference was significant ($P < 0.05$) between the 48th dpi and the 7th, 14th, 21st, and 28th dpi groups.

Blood glucose: Measurement of blood glucose levels showed a significant difference between groups. The lowest value was determined in the 21st dpi group (83.6±3.05) & 28th dpi (95.6±5.08) with significant differences ($P < 0.05$) as compared to all other infected groups & negative control (125±8.61), followed by significant elevations in blood glucose started from the 35th dpi and reached highest value on the 48th dpi (138.2±2.17). Difference between the 48th dpi group was statistically significant ($P < 0.05$) when compared to negative control, 7th, 14th, 21st, and 28th dpi groups.

Serum insulin: There was a significant difference between groups as to serum insulin levels. In normal control (NC) insulin level was 6.78±0.15 followed by significant increases started from 7th dpi, reached highest value on the 21st dpi (8.7±0.34) and then gradual decrease in serum insulin was determined started from the 28th dpi and reached the lowest value in the 48th dpi (5.72±0.55). Difference between the 48th dpi group was significant ($P < 0.05$) when compared to the NC, 7th, 14th, 21st, and 28th dpi groups.

Histopathological study of skeletal muscle tissues showed normal architecture in NC and the 7th dpi mice groups. On the 14th dpi, very small larvae were surrounded by few inflammatory cells. Sections from infected mice on the 21st dpi showed areas of severe muscle damage around many *T. spiralis* larvae. Each larva was enclosed by a collagenous capsule inside a nurse cell. Nurse cells were surrounded by inflammatory cells. By the 28th dpi, most of the nurse cells were completely developed and increased their sizes with signs of skeletal muscles degeneration such as basophilic cytoplasm and loss of muscle bundle striation. On the 35th dpi, aggregates of inflammatory cells were seen at the poles of the nurse cells capsule. Then,

on 48th dpi, there were intense inflammatory infiltrates around nurse cells and between muscle bundles. Also, severe blood congestion was seen in this group.

Glycogen accumulation in infected muscle cells was investigated using PAS. Strong staining denoting high glycogen accumulation was observed in cytoplasm of *T. spiralis* infected muscle on the 7th, 14th, 21st, and 28th dpi but, less PAS staining showed glycogen depletion in *T. spiralis*-infected muscle cells on the 35th dpi and the 48th dpi.

Immunohistochemical localization of irisin in skeletal muscle sections from mice of normal control and 7th dpi showed negative and /or sporadic expressions. On 14th dpi, there were strong expressions inside muscle bundles and inflammatory cells. On 21st, 28th and 35th dpi there were moderate to strong exp-r-essions surrounding *T. spiralis* larvae and in the inflammatory infiltrate. On 48th dpi, intense irisin expression was observed around nurse cells, in adipose tissues, in the inflammatory infiltrate, and in nerve bundles innervating the skeletal muscles that showed moderate expression.

Details were given in table (1) and figures (1, 2, 3, 4 & 5).

Table 1: Mice body weight, blood glucose and serum insulin levels among groups.

Studied group (n = 5)	Body weight (g)	Blood glucose (mg/dl)	Serum insulin (µIU/ml)
NC	24.46±1.48	125±8.61	6.78±0.15
7th dpi	24.04±1.61	104.2±8.26 [a]	8.26±0.41 [a]
14th dpi	23.46±0.73	105.2±5.81 [a]	8.34±0.27 [a]
21st dpi	23.24±0.6	83.6±3.05 [a b c]	8.7±0.34 [a]
28th dpi	23.32±0.78	95.6±5.08 [a d]	7.64±0.64 [d]
35th dpi	21.6±2.2 [a b]	132.6±8.29 [b c d e]	6.2±0.47 [b d e]
48th dpi	20.4±0.96 [a b c d e]	138.2±2.17 [a b c d e]	5.72±0.55 [a b c d e]
ANOVA	F= 5.94	F= 50.46	F= 36.5
P-value	P= 0.000	P= 0.000	P= 0.000

[a]significance compared to NC, [b]significance compared to 7th dpi, [c]significance compared to 14th dpi, & [d]significance compared to 21st dpi, [e]significance compared to 28th dpi, and [f]significance compared to 35th dpi.

Discussion

Trichinella spiralis is a parasitic nematode that infects striated muscle cells of a variety of mammalian hosts and causes one of the most important food-borne zoonotic diseases causing hypoglycemia due to high gluco-se consumption by the rapidly growing larvae (Wu *et al*, 2009). These growing larvae were found to depend on insulin signaling pathways to supply the needed glucose and to convert glucose into stored glycogen molecules (Youngren, 2007). But, the exact regulatory mechanisms of glucose metabolism during trichinosis remain still unclear. Irisin is a recently discovered myokine secreted by skeletal muscles during exercise to increase energy expenditure by stimulating glucose uptake and the accumulation of glycogen in muscle cells (Yano *et al*, 2021).

In the present work, changes in mice body weight, blood glucose level, serum insulin, histopathological alterations, glycogen storage, and the immunohistochemical expression of irisin in skeletal muscles of experimentally infected mice with *T. spiralis* were evaluated.

There was a significant difference between the studied groups. A gradual insignificant reduction of body weight over time after *T. spiralis* infection was observed. This change in body weight started from the 7th dpi and continued till the 28th dpi then on the 35th and 48th dpi, the reductions were statistically significant. This agreed with Dlugosz *et al*. (2013), who reported a decrease in body weight of *T. spiralis*-infected mice starting from the 14th dpi. Also, results agreed with Kang *et al*. (2021), *T. spiralis* infection ameliorated diet-induced obesity in a mouse model. They added that food intake was not affected by parasitic infection, suggested another mechanism involved in this weight loss such as M2 macrophage proliferation and/or changes in gut microbiota. However, Marian *et al*. (2020) noted a slight insignificant decrease in body weight of *T. spiralis*-infected mice groups after the 7th dpi. Also, Wang *et al*. (2021) noticed initial decreases till the 6th

and 9th dpi, then gradual weight gain till the 15th dpi in mice infected with *T. spiralis*. However, Okada *et al*. (2013), didn't find changes in body weight.

Loss of body weight associated with the establishment of *T. spiralis* larvae in skeletal muscles could be contributed to anorexia which is a common effect of infection in general. Moreover, *T. spiralis* infection was found to induce a significant increase in serum TNF-α in correlation with a period of hypophagia during nurse cell development and myositis (Worthington *et al*, 2013). Additionally, the systemic release of cytokines such as IL-1β, TNF-α, and IL-6 was reported to stimulate leptin secretion by adipocytes to reduce hunger and food intake (Yu *et al*, 2013).

In the present study, the blood glucose levels showed a significant difference between groups. The lowest values were determined on the 21st and 28th dpi with significant differences when compared to all others. This reduction was followed by significant elevations in blood glucose starting from the 35th dpi and reached the highest significant value on the 48th dpi. This agreed with Wu *et al*. (2009), who reported transient insignificant hypoglycemia between the 8th and 28th days post-*T. spiralis* and *T. pseudospiralis* infections. They claimed this hypoglycemia due to the increased glucose consumption by the growing larvae, the reduced absorptive ability of the intestine, or the impaired production of glucose by the liver in addition to the increased expression of insulin signaling factors. On the contrary in their experiment, during the late stage of *T. spiralis* infection (28th & 48th dpi), blood glucose level and expression of insulin signaling factors returned to normal, suggesting that glucose uptake after nurse cell formation could be dependent on different mechanisms from those at the early stage.

The present study was in coincidence with *T. spiralis* established larvae in the skeletal muscles of highly infected groups, peak blood glucose concentrations were recorded from the 35th to the 49th dpi (La Grange and Mukaratirwa, 2014). Also, a significant increase in blood glucose concentration was on the 30th dpi in pigs (Oltean *et al*, 2012).

Generally, the regulation of blood glucose levels is dependent on insulin released from β-pancreatic islet cells. High blood glucose stimulates pancreatic islet cells to secret insulin which enhances glucose uptake by insulin-responsive tissues (e.g. skeletal muscle and adipose tissue) leading to hypoglycemia (Wu *et al*, 2009).

In normal muscle bundles, the movement of glucose through cell membranes occurs via glucose transport proteins (GLUT-1 and 4). So, muscle fibers are highly sensitive to insulin, which increases glucose transport and glycogen accumulation (Lee *et al*, 1997).

During early *T. spiralis* infection, larvae utilized the host metabolic system for their benefit by increasing insulin levels to facilitate glucose uptake, and, insulin signaling pathway-related genes, such as insulin receptor (IR), insulin receptor substance-1 (IRS-1), and IRS-2 were found up-regulated in nurse cells (Wu *et al*, 2013). While in late infection, insulin have a minor effect on glucose uptake rate by the isolated nurse cells on the 40th dpi in an *in vitro* study (Montgomery *et al*, 2003). They claimed this poor response to insulin to the collagenous capsule of nurse cells which physically hinders insulin receptor docking or due to the loss of functioning insulin receptors. Thus, glycogen synthesis depended on other factors such as host immune response, species, sex, age, and nutritional conditions (Sidor and Andreyanov, 2020).

Regarding serum insulin levels in the present work, there was a statistically significant difference between the studied groups. The results revealed significant increases starting from the 7th dpi and the highest value was measured on the 21st dpi, then gradual decreases were detected starting from the 28th dpi and the lowest significant value was detected on the 48th dpi. This agreed with the reported of increase in insulin concentra-

tion in *T. zimbabwensis* infected mice (Onkoba *et al*, 2016; Murambiwa *et al*, 2022).

On the contrary, Wu *et al*. (2009) observed hypoinsulinemia in *T. spiralis*-infected mice on the 8th and the 18th dpi when compared to uninfected mice then it returned to normal values. This controversy could be assumed to using different mice models. In the study conducted by Wu *et al*. (2009), nude mice were used while Swiss albino mice were utilized in the current work. Nude mice are naturally mutated mice that lack thymus gland and hair. The absence of thymus gland makes these mice immunodeficient due to the deficiency of T-cells (Szadvari *et al*, 2016). In this context, Li and Ko (2001) observed an absence of cellular infiltrates in *T. spiralis*-infected nude mice; so, they claimed that the inflammatory response during trichinosis is T-cell dependent. Furthermore, a close interaction between immune and endocrine systems especially concerning glucose metabolism regulation was documented (Wensveen *et al*, 2019). For example, IFN-γ secreted during infection in general was found to inhibit insulin sensitivity in skeletal muscle via downregulating insulin receptors leading to reactive hyperinsulinemia (Šestan *et al*, 2018). In the case of *T. spiralis* infection in experimental mice, mRNA expression of IFN-γ was upregulated at all phases of the disease (Yu *et al*, 2013).

In the present study, skeletal muscle tissues on the 14th dpi, the larvae appeared inside muscle bundles surrounded by few inflammatory cells. On the 21st dpi, areas of severe muscle damage around many *T. spiralis* larvae were seen. The larvae were enclosed by a collagenous capsule within the developing nurse cells and surrounded by inflammatory cells. By the 28th and 35th dpi, most of the nurse cells were completely developed and increased in size with aggregates of inflammatory cells at their poles. Finally, on the 48th dpi, intense inflammatory infiltrates around nurse cells containing mature larvae were observed. Also, inflammatory cellular reactions were seen between muscle bundles, and severe blood congestion with foci of hemorrhage were noted. These histopathological findings were in accordance with previous studies conducted on *T. spiralis* infection in experimental mice (Boonmars *et al*, 2005; Othman *et al*, 2016; El-Aswad *et al*, 2020; Fadil *et al*, 2022).

Regarding glycogen accumulation in the infected muscle cells assessed by PAS stain, the results revealed strong staining signifying high glycogen accumulation in *T. spiralis*-infected muscle on the 7th, 14th, 21st, and 28th dpi Whereas, less PAS staining denoted glycogen depletion was observed on the 35th and 48th dpi. This agreed with Wu *et al*. (2009) who found an association between low blood glucose levels and the increased glycogen content in *T. spiralis* infected muscles during the period from the 4th to 28th dpi. They stated that the larvae continued to actively accumulate glycogen for one and a half months then from the 45th to 134th dpi, the intensity of glycogen storage significantly decreased. However, some studies documented this phenomenon as early as the 28th dpi (Sidor and Andreyanov, 2020). Also, Montgomery *et al*. (2003) and Okada *et al*. (2013) reported that the larvae after invasion into muscle bundles started from the 7th dpi to accumulate glucose and synthesize glycogen in the nearby cells to be consumed in the subsequent intensive larval organogenesis.

Concerning the immunohistochemical localization of irisin in skeletal muscle sections from *T. spiralis* infected mice, on the 14th and 21st dpi there were strong irisin expressions inside muscle bundles. On the 28th and 35th dpi, there were moderate to strong expressions surrounding *T. spiralis* larvae and in the inflammatory infiltrates. On the 48th dpi, intense irisin expression was around nurse cells, in adipose tissues, in inflammatory infiltrate, and nerve bundles innervating skeletal muscles with less expression in muscle bundles. *In vitro,* treatment of myocytes with irisin for different durations increased glycolysis and oxidative metabolism via increasing the expression of several genes in-

volved in the metabolism like glucose transporter (GLUT-4) and mitochondrial uncoupling protein 3 (UCP-3) (Vaughan *et al*, 2014). The current study suggested that the increased irisin expression during the early stage of nurse cell development (14^{th} & 21^{st} dpi) increased insulin responsiveness of *T. spiralis* infected skeletal muscles, facilitated glucose transport via GLUT-4 transporter, resulted in glycogen accumulation in muscle bundles and larvae. So, by irisin, *T. spiralis* infected skeletal muscles communicated with the pancreatic islets to regulate insulin secretion and glucose metabolism for their benefit and growth. Moreover, the increased irisin expression in the present work correlated with the kinetics of blood glucose which showed the lowest significant values on the 21^{st} and 28^{th} dpi. A decrease in the intensity of irisin expression inside muscle bundles starting from the 28^{th} dpi in coincidence with the maturation of nurse cells was observed in this study, while the expression was strong in the inflammatory infiltrate. On the 48^{th} dpi, strong irisin expressions were noticed in nerve bundles, adipose tissues, and the inflammatory cell aggregates denoting another role of irisin during *T. spiralis* infection rather than the metabolic functions supplying the growing larvae with their nutritional demands. Aydin *et al.* (2014) reported that Irisin expression in nerve sheaths and skeletal muscle sarcoplasm. Also, irisin was found to be secreted by adipose tissues to act as adipo-myokine (Kirat *et al*, 2021) The increased irisin expression in mice caused weight loss suggested that irisin may be involved in different compensatory mechanisms for metabolism regulation (Chen *et al*, 2016). So, in the present study, irisin may have a possible role in significant loss of mice body weight during the period from the 35^{th} to 48^{th} dpi. Also, irisin has some prote ctive, antioxidant, and anti-inflammatory activities in metabolically stressed muscles (Vaughan *et al*, 2014; Zhu *et al*, 2015; Bosma *et al*, 2016). The irisin protective and anti-inflammatory role against cytokine-induced cellular damage stimulated by AMP-activated protein kinase (AMPK) pathway (Alves *et al*, 2022). This stimulated pathway reduces the production of inflammatory mediators such as COX-2, iNOS, IL-1β, IL-6, and TNF-α (Jiang *et al*, 2021). Noteworthy, these molecules are key players during the inflammatory immune response to *T. spiralis* infection (Scales *et al*, 2007).

Controlling macrophage differentiation could be another mechanism of the suggested anti-inflammatory role of irisin during the late stage of *T. spiralis* infection in the present work. Macrophages are classified into two types, classically activated (M1) macrophages and alternatively activated (M2) macrophage types (Van den Bossche *et al*, 2016). M1-type secretes pro-inflammatory cytokines such as TNF-α and IL-1β while M2-type produces anti-inflammatory cytokines such as IL-10 (Van den Bossche *et al*, 2016). Regarding this process of macrophage polarization, irisin was found to play a significant role. For example, exogenous FNDC5 inhibited lipopolysaccharide (LPS)-induced M1-type macrophage differentiation whereas its deficiency enhanced LPS-induced M1-type macrophage differentiation (Xiong *et al*, 2018; Slate-Romano *et al*, 2022).

Conclusion

The increased irisin expression in muscle bundles increased insulin responsiveness of *T. spiralis*-infected skeletal muscles to facilitate glucose transport and accumulate glycogen essential for larval growth in the early stage of infection. However, during the late stage, irisin affected fat metabolism and contributed to loss of body weight. Increased irisin expression in the inflammatory infiltrated have protective, antioxidant, and anti-inflammatory roles.

Host-parasite endocrine relationship is a complicated process, and many aspects not discovered yet. So, studies are ongoing to clarify other molecules and hormones inolved in this interplay and to identify irisin exact role in trichinosis management and will be

published in due-time

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Authors' Contribution: The authors equally contributed in theoretical and practical study.

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Explanation of figures

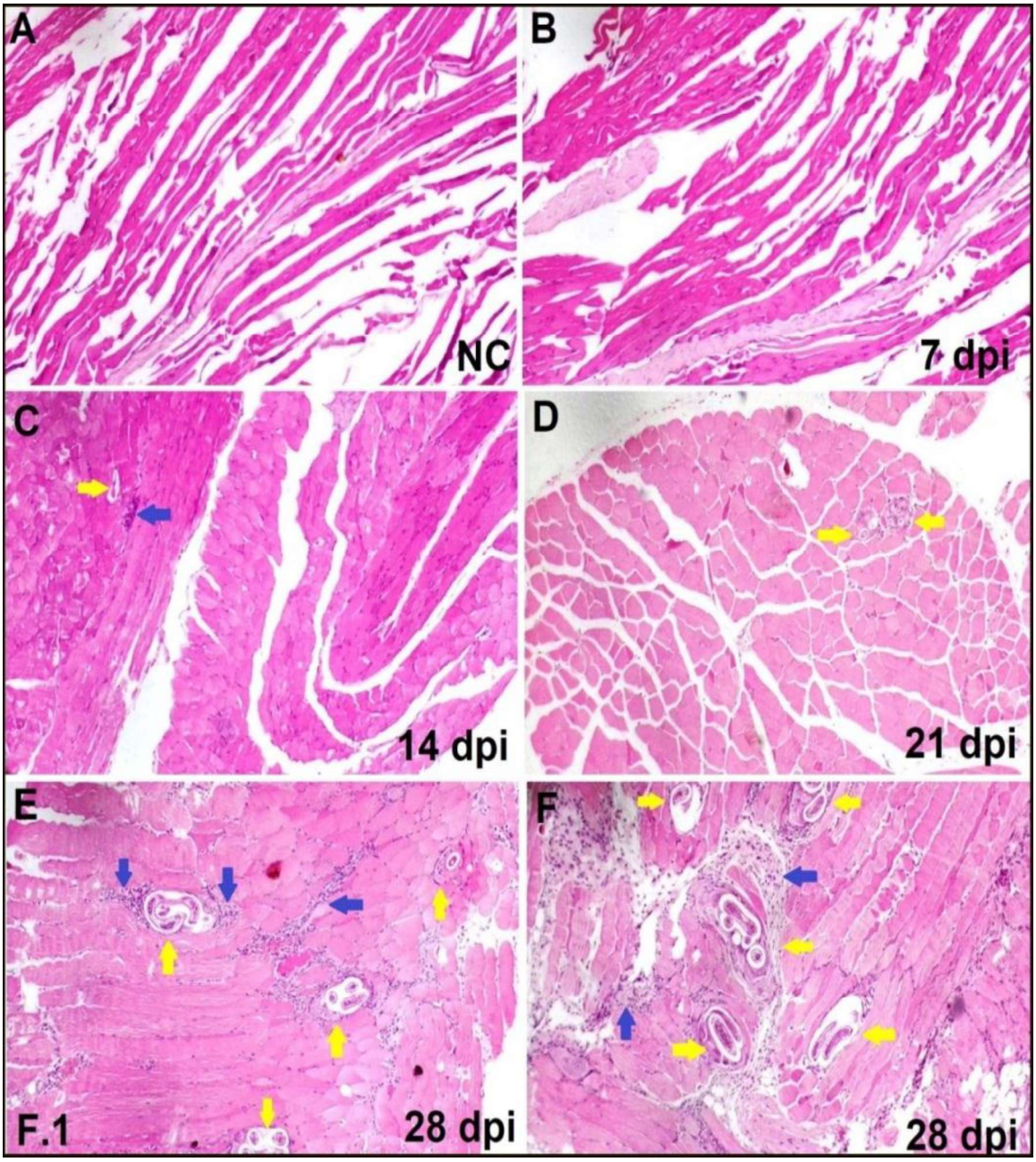
Fig. 1: H. & E.-stained skeletal muscle sections from mice of A) normal control (NC) group & B) 7 dpi showed normal histological structure. C) 14 dpi and D) 21 dpi showed *T. spiralis* larva (yellow arrow) inside muscle bundles and inflammatory infiltrate (blue arrow). E) & F) from 28 dpi showed numerous larvae inside well-developed capsules surrounded by moderate inflammatory infiltrate and degenerated or hyalinized muscle fibers (x40).

Fig. 2: H. & E.-stained skeletal muscle sections from mice of A) and B) 35 dpi group showed few uninfected muscle bundles (um) and numerous infected muscle bundles with *T. spiralis* larvae (L) enclosed inside collagenous capsules (black arrows) and nurse cells (red arrows) characterized by basophilic cytoplasm (b) and surrounded by moderate inflammatory infiltrate (blue arrows) (x40). C), D) and E) 48 dpi showed several well-developed nurse cells and *T. spiralis* larvae (L). Intense inflammatory infiltrates around nurse cells and between muscle bundles (blue arrows, black rectangle) and dilated congested blood vessels with foci of hemorrhage (yellow stars) (x40). F) Higher magnification of black rectangle showed intense lymphocytic infiltrate (x100).

Fig. 3: PAS-stained skeletal muscle sections from mice of A) normal control (NC) group and B) 7 dpi showed normal histological structure and glycogen storage (g) (x40). C) 14 dpi *T. spiralis* larva (yellow arrows) inside muscle bundles (x40). D) 21 dpi and E) 28 dpi showed *T. spiralis* larva (L) and increased glycogen (g) inside muscle bundles and in the larva (x40). F) 35 dpi showed *T. spiralis* larva (L) and glycogen (g) started to decrease in muscle bundles (x100). G) (x40) and H) (x100) from 48 dpi showed numerous well-developed larvae (L) and decreased glycogen storage (g) inside muscle bundles and larvae.

Fig. 4: Immunohistochemical localization of irisin in skeletal muscle sections from mice of A) normal control (NC) group & B) 7 dpi showed negative to sporadic expression (x40). C) 14 dpi showed strong expression (x40). D) 21 dpi, E) 28 dpi and F) 35 dpi showed moderate to strong expression surrounding T. *spiralis* larvae (red arrows) in inflammatory infiltrate (blue arrows) (x40).

Fig. 5: Immunohistochemical localization of irisin in skeletal muscle sections from mice of 48 dpi showed numerous *T. spiralis* larvae (red arrows) and strong irisin expression (blue arrows) (x40) A) in nerve bundles B) in inflammatory infiltrate in between muscle bundles and nerve bundles C) in inflammatory infiltrate surrounding nurse cells D) in adipose tissues and inflammatory infiltrate in between the infected muscle bundles which showed moderate expression.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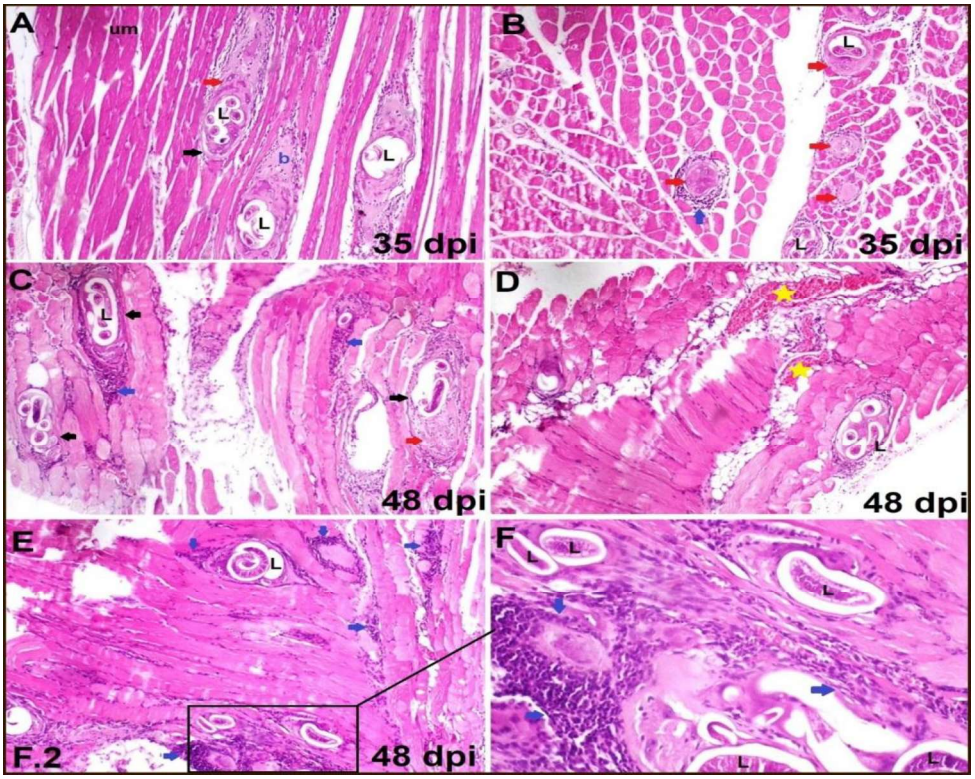
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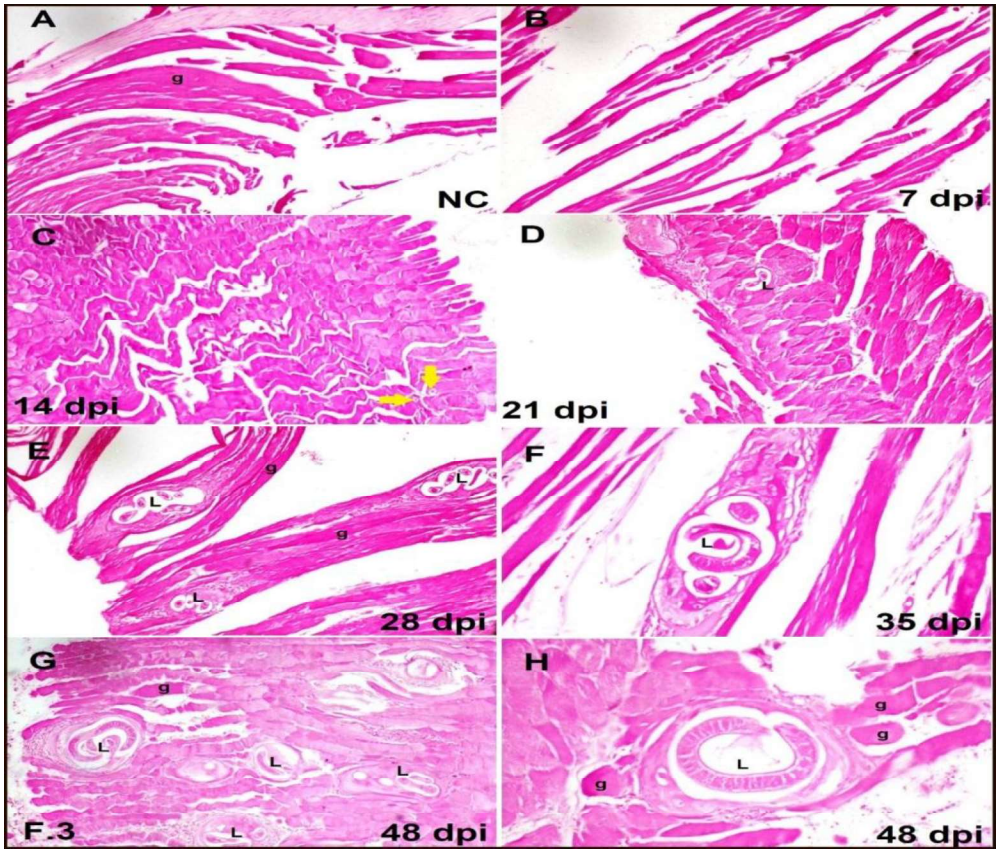
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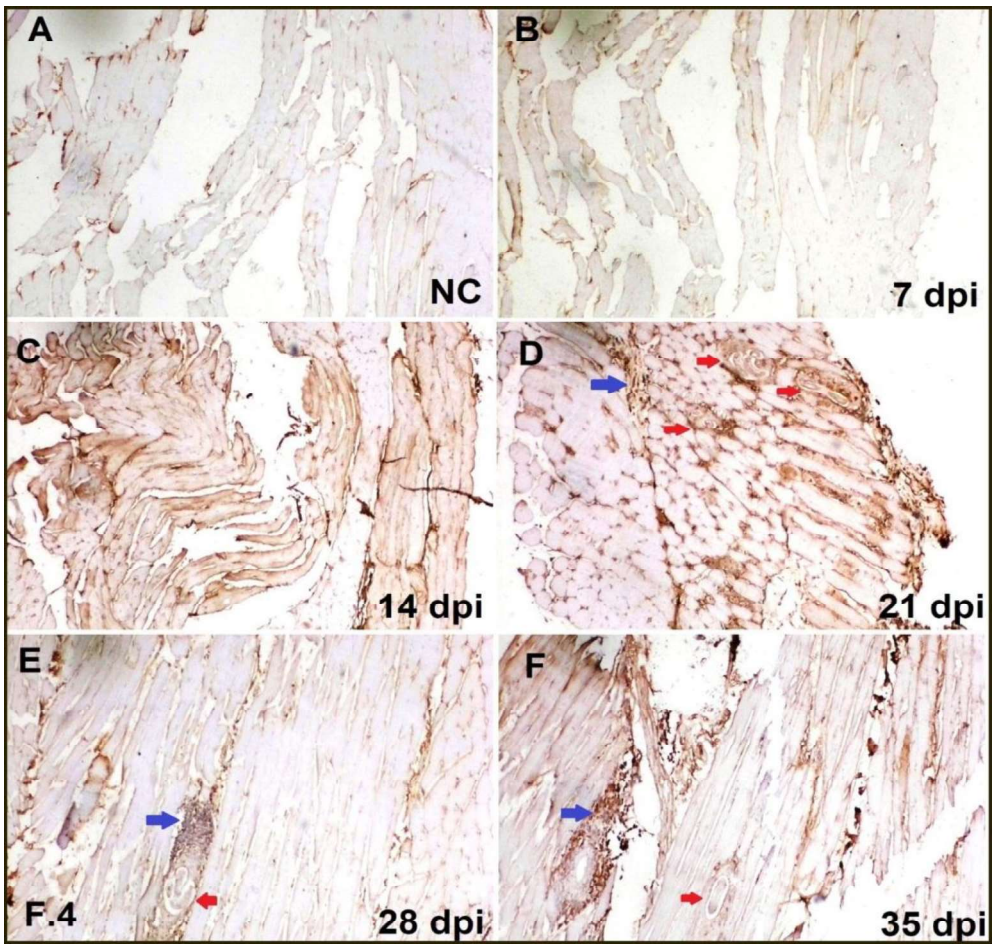
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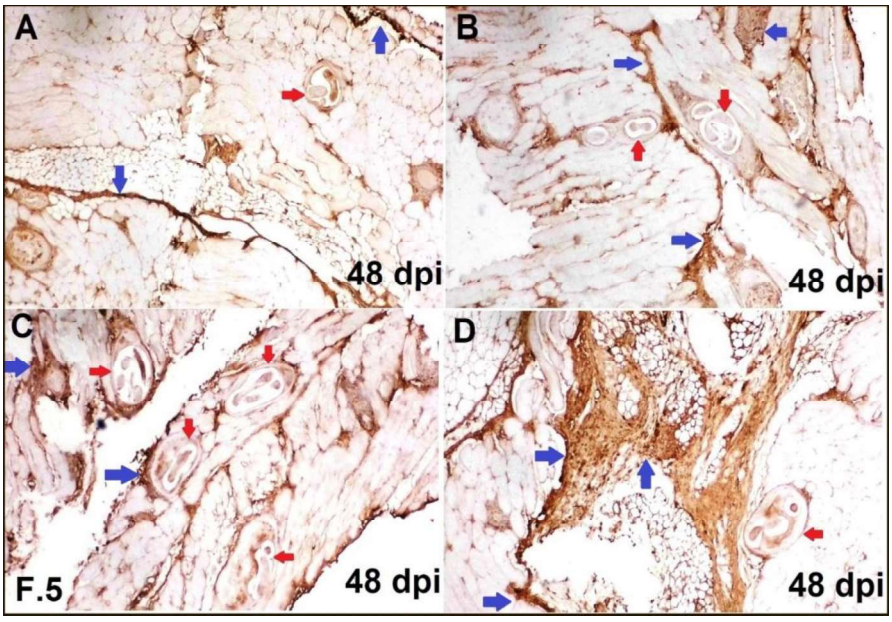
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