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Original Research

Investigation of the irisin, preptin and adropin levels in the blood serum of *Alburnus tarichi*

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Abstract: Irisin, preptin and adropin are three newly discovered peptides that play critical roles in regulating energy homeostasis in various vertebrates.The purposes of this study were to measure the serum concentrations of preptin, adropin and irisin in the *Alburnus tarichi* and to investigate the relationship of these peptides to the weight, gender and length of this the fish, which will provide useful information for future biotechnology purposes aimed at improvements in aquaculture production. This study used 12 adult *A. tarichi* (6 female and 6 male) obtained from Van Lake (Van, Turkey). The serum irisin, preptin and adropin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit to determine correlations between the levels of these three hormones and fish body weight and length. No statistically significant correlations were detected between the serum irisin, adropin and preptin levels and *A. tarichi* body weight ($p = 0.921$, $r = -0.031$; $p = 0.08$, $r = 0.519$; $p = 0.461$, $r = -0.235$, respectively) or length ($p = 0.901$, $r = -0.040$; $p = 0.105$, $r = 0.490$; $p = 0.236$, $r = -0.369$, respectively).Thus, serum levels of these hormones are independent of fish gender, body weight and length.

Key words: *A. tarichi*; Irisin; Preptin, Adropin, Metabolic hormone.

Introduction

The term "energy homeostasis" refers to metabolic events involving energy expenditure and digestive behaviour (1). In fish, energy homeostasis, feeding behaviour and food intake are regulated by the interaction of the hypothalamus and peripheral endocrine pathways that respond to the body's energy status and requirements (2). Some newly discovered metabolic hormones that maintain energy homeostasis in fish are preptin, adropin and irisin (3,4). The levels of thesenovel peptide hormones are regulated by nutrients, including proteins, lipids and carbohydrates.

Irisin, a glycoprotein hormone, was first isolated from muscle tissue by Boström et al. (5). It is 12 kDa in size and consists of 112 aminoacids. Current research indicates that irisin is synthesised by various tissues, with its main source being skeletal muscle and adipose tissue. It is also found in human milk, saliva and various biological liquids (3).The structure of the irisin hormone is 100% similar in humans and mice (6).

Adropin is synthesised mostly in the brain and liver, through expression of the Energy Homeostasis Associated Gene (Enho) (7, 8, 9), although it is also found in various peripheral tissues (7). It consists of 76 aminoacids and has a molecular weight of 4499.9 Da. Adropin is defined as an independent factor that preserves insulin resistance (10). It also plays a significant role in lipid metabolism, in maintaining insulin sensitivity and in energy homeostasis (11). Preptin, a peptide first explored in 2001, is predominantly synthesised in beta cells in response to postprandial glucose levels. It mediates physiological insulin secretion due to glucose concentration (12). Preptin also participates in regulating carbohydrate, lipid, and protein metabolism (12,13), a function also shared by adropin (7). By contrast, irisin regulates adipose tissue metabolism and glucose homeostasis by its synthesis in peripheral tissues, including adipose tissues, salivary glands, kidneys and liver, but predominantly in cardiac muscle (3, 5).

Most of the recent research on these hormones has been conducted on mammals (5, 7, 12, 13), so little information about these new peptide hormones is available for fish.The aim of the present research was to provide data about irisin, preptin and adropin in terms of the weight, gender and length relationships in fish, focusing on the Cyprinidae, an important family of fish with worldwide distribution. In Turkey, the family has approximately 30 genera and 70 species (14, 15). One economically important species in Turkey fisheries is the *Alburnus tarichi*, a unique fish species that only inhabits the Van Lake basin (16). In this study, we have investigated the body weight, length and gender in *A. tarichi* in terms of serum levels of these three new metabolic hormones. Our goal is to provide new information that will be useful in future biotechnology studies aimed at improving aquaculture production of this fish.

Materials and Methods

This study investigated 12 adult *A. tarichi* (6 female and 6 male) obtained from Van Lake (Van, Turkey).The

length and weights of each fish were measured and the animals were then anaesthetized with 0.1% concentration of tricaine methane-sulfonate. Blood wasthen drawn from the caudal vein, the blood samples were placed in aprotin in tubes to prevent denaturation of the proteins and the samples were stored at -80 °C until analyses. Irisin, adropin and preptin levels were determined using ELISA methods (Irisin: Phoenix Pharmaceuticals, Belmont, CA, USA, Cat.No. EK-067-16; Preptin: MyBioSource San Diego, California, USA,Cat. No. MBS108945;Adropin: MyBioSource San Diego, California, USA,Cat.No. MBS092568).The intra- and inter-assay coefficients of variation and sensitivity for irisin were 5.61 % and 14.56 %, respectively. The intra- and inter-assay coefficients of variation and sensitivity for preptin and adropin are less than 15%. The plates were read at 450 nm with a plate reader (Spectra Max Plus 384, Molecular Devices LLC, Sunnyvale, CA). The existing literature indicates that these hormones have been analysed previously by mRNA expressionusing the polymerase chain reaction (PCR); however, the protein expression does not entirely reflect the hormone levels in the circulation. The ELISA method was implemented here to determine the levels of these hormones for economic reasons, its availability, its usefulness for even small concentrations and its quick results (17).

Statistical Analysis

Values are expressed as means ±SE. The Kolmogorov–Smirnov Z test showed that the data were not normally distributed. The Mann-Whitney U test, which is a non-parametric comparison, was used to analyse the significance of the comparison between the sexes in this species. Irisin, adropin, preptin level and physical characters (body weight, and length) of each fish species was comparedusing Spearman correlation. A value of $p < 0.05$ was accepted as statistically significant, and $p < 0.0001$ was accepted as highly statistically significant.

Results

In this study, the mean length and weight of *A. tarichi* were measured as 22.12 ± 1.4 cm and 88.63 ± 14.3 g, respectively. The variations in individual irisin, adropin and preptin serum values for *A. tarichi* are shown in figures 1, 2 and 3, respectively.

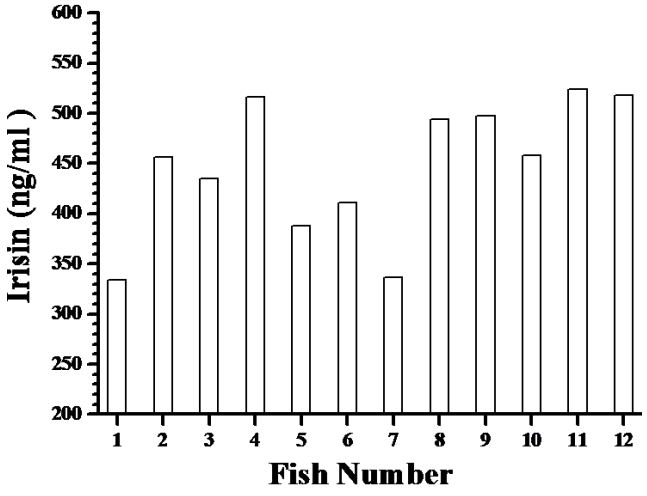

Figure 1. Individual irisin variation values of *A. tarichi.*

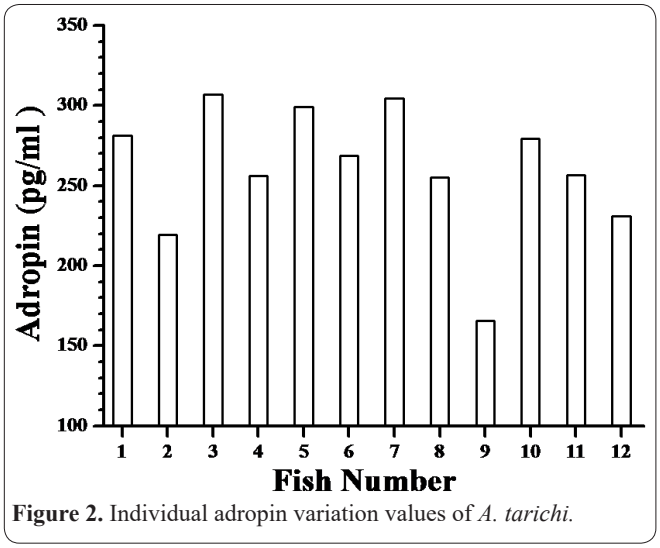

Figure 2. Individual adropin variation values of *A. tarichi.*

Irisin levels measured in the male (n=6) (415.5±27.8 ng/mL) and female (n=6) (479.2±22.6 ng/mL) *A. tarichi* did not differ significantly ($p > 0.05$) (Figure 4). Adropin levels in the male (n=6) (253.02±20.9 pg/mL) and female (n=6) (287.35±11.9 pg/mL) *A. tarichi* also showed no significant differences ($p > 0.05$) (Figure 5). Similarly, preptin levels measured in the male (n=6) (13.79±1.0 ng/mL) and female (n=6) (13.02±0.5 ng/mL) *A. tarichi* also showed no significant differences ($p > 0.05$) (Figure 6).

No significant correlations were identified between

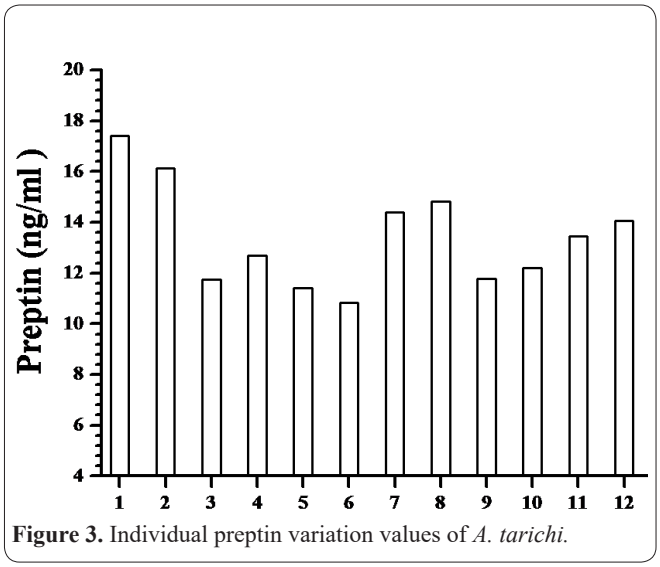

Figure 3. Individual preptin variation values of *A. tarichi.*

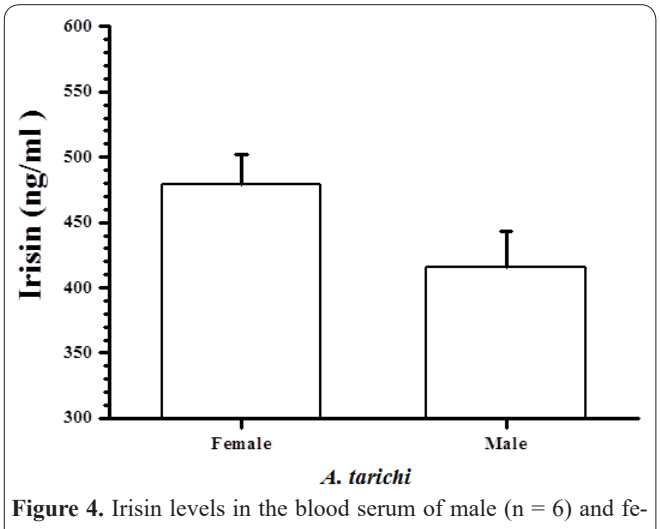

Figure 4. Irisin levels in the blood serum of male (n = 6) and female (n = 6) of *A. tarichi* (mean ± SE, $p < 0.05$).

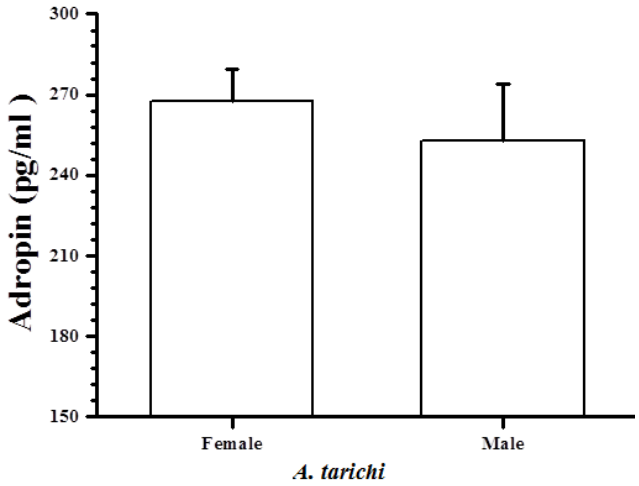

Figure 5. Adropin levels in the blood serum of male (n = 6) and female (n = 6) of *A. tarichi* (mean ± SE, p < 0.05).

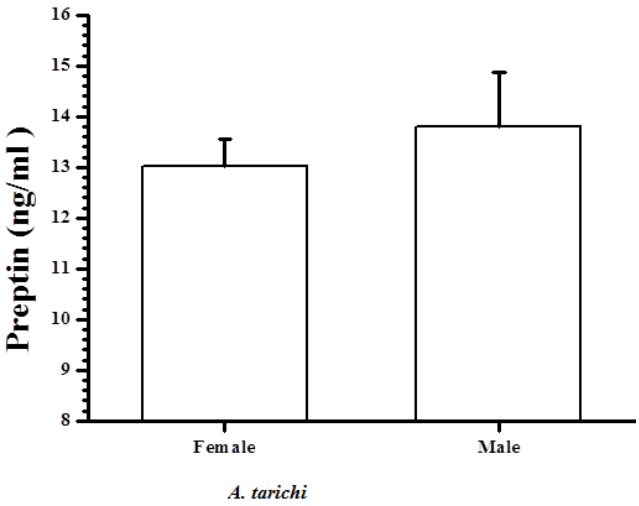

Figure 6. Preptinlevels in the blood serum of male (n = 6) and female (n = 6) of *A. tarichi* (mean ± SE, p < 0.05).

the irisin, adropin and preptin levels in the blood serum and the fish body weight (p = 0.921, r= -0.031; p = 0.08, r = 0.519; p = 0.461, r = -0.235, respectively) or length (p = 0.901, r = -0.040; p = 0.105, r = 0.490; p = 0.236, r = -0.369, respectively).

Discussion

Previous studies on metabolic hormones in fish identified ghrelin (18), apelin (19), nesfatin (20, 21, 22)and leptin (23, 24). However, little information exists regarding adropin, irisin, and preptin, or the differences in their concentrations (if any), in fish. The normal values of adropin, preptin and irisin, which play significant roles in energy regulation, are not known for fish circulation. In this study, the presence of all three hormones was confirmed in the blood of *A. tarichi* and the serum levels were examined in terms of body weight and gender in this fish.

Irisin has been studied in various tissues and biological liquids in mice (5), humans (25), chickens (26) and rats (27), where itis reported to play a role in the regulation of various physiological events. Irisin decreases preadipocyte differentiation in adipocytes, prevents lipid accumulation in fat cells and accelerates muscle hypertrophy in humans (25). Li et al. (26), in their study on chickens, confirmed that FNDC5/irisin was more than one myokine and was involved in various physiological processes, such as growth of white matter andregulation of muscular and adipose tissue functions, in addition to energy metabolism. However, information is scarcer regarding the function and structure of irisin in fish. In zebra fish, irisin is reported to palliate embolisms chemically (28). FNDC5/irisin mRNA was expressed in all tissues examined, although the relative abundance varied. In tilapia, FNDC5 mRNA expression was located in the optic tectum, spinal cord and telencephalon, with relatively high levels in the cerebellum, hypothalamus, medulla oblongata and pituitary, lower levels in mesenteric fat, liver and muscle, and low levels in the intestine, kidney, spleen and stomach (29). In the same study, Lian (29) also used primary cultures of tilapia pituitary cells as a model to investigate the pituitary actions of irisin for regulation of growth hormone gene expression and secretion and the signal transduction pathways regulating irisin-inhibited growth hormone mRNA expression. Butt et al. (30) reported that irisin injections decreased food intake and that irisin can be an important regulator of feeding and energy homoeostasis in goldfish.

In the present study, the presence of irisin was confirmed in the serum of *A. tarichi.* Irisin levels did not differ between the sexes in *A. tarichi*. Similarly, no correlation was found between serum irisin levels and *A. tarichi* body weight. Studies on mammals have revealed that the level of plasma irisin is not directly related to changes in body weight, but it does show a correlation with basal energy expenditure (31). Previous studies suggested a correlation between irisin and BMI (32, 33).

Irisin, which is stimulated by exercise and cold, increases the expression of the mitochondrial pump, termed uncoupling protein 1 (UCP1), found in white adipose tissues. The increase in UCP1 expression is coupled to increased heat generation in the cell, thereby maintaining thermogenesis and glucose homeostasis (34). Irisin, which is defined as a thermogenic peptide, executes the transformation of white adipose tissue to brown adipose tissue through increases in FNDC5 UCP1 expression (17). In mammals, UCP1 is only found in brown adipocytes, although the latest evidence indicates a low-level expression in thymocytes (35). On the contrary, UCP1 is found in fish (tilapia) mainly in the liver (29).UCPs are located on the mitochondrial inner membrane and play a part in various physiological processes, such as ATP generation, mitochondrial anion transporter protein function, insulin secretion, glucose and lipid metabolism, adaptive thermogenesis, mitochondrial biogenesis, synaptic transmission, neuronal differentiation, neuronal degeneration, ROS generation and hormone secretion (34).

Several studies have examined the physiology of adropin in mammals. For example, a negative correlation was observed between plasma adropin concentrations and hunger triglyceride levels(10). Plasma adropin concentration also showed a correlation with endothelial dysfunction (9). The plasma levels of adropin are also elevated in patients with coronary failure (36). By contrast, the physiological significance of adropin in fish is still not well understood. The amino acid sequence of the adropin protein is100% similar in humans, mice

and rats (8). However, while the aminoacid sequence of tilapia adropin shares relatively high levels of similarity with that of platyfish(87%), mollies (82%) and sticklebacks (82%), the similarity level to adropin in chickens (46%), crocodiles (44%), cows (36%), mice (35%) and humans (35%) is quite low (37). Real-time polymerase chain reaction studies show that tilapia adropin transcripts are specific to the liver and hypothalamus, with only low levels of expression seen in peripheral tissues (37). In the present study, adropin was confirmed to exist in the serum of *A. tarichi*, but had no correlation with fish gender or body weight.

Preptin, a 34 amino acid peptide, is a derivative of proinsulin growth factor II (pro-IGF-II), secreted by pancreatic β cells and is considered a physiological enhancer of insulin secretion. Preptin has a stimulating effect on osteoblasts, inducing their proliferation, differentiation and survival. Notably, preptin also has an anabolic effect on bone tissue, which might indicate a preventive role in osteoporosis (38).The amino acid sequence of human preptin shows 79.41% similarity with that of mouse preptin, while the similarity rate is 73.53% with rat preptin. (The similarity between mouse and rat preptin aminoacid sequences is 94.12%.) ELISA study results indicated that the normal serum preptin concentration in human varied between 7.9±1.35 ng/mL and 10.11 ±1.61 ng/mL, while in human milk it varied between 9.72 ± 2.26 ng/mL and 14.32±3.06 ng/mL (3). A study of normal-weight individuals showed that the circulating level of preptin was 398 ±13ng/L and that preptin levels were lower in males than females (39). The normal concentration of preptin in the fish circulatory system is still unknown.The present study is the first in the literature to evaluate preptin levels in terms of fish length, gender and body weight. Preptin was confirmed to exist in the serum of *A. tarichi,* but the levels did not show any significant correlation with gender or body weight in this fish.

We have determined that no significant difference exists in the level of these three hormones according to gender in *A. tarichi.* In addition, no correlation exists between the weight and length of this fish and the irisin, preptin and adropin levels. The results of this study demonstrate that the serum levels of these hormones, which play an important role in energy regulation, are independent of fish gender, body weight and length. These data contribute important information that can support more comprehensive studies and further illuminate the knowledge gained in earlier studies on the subject of hormonal regulation in fish.

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N/A. Experimental animals were handled in accordance with national and international guidelines for the protection of animal welfare.

Interest conflict
There is no conflict of interest.

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