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Research Article

Determination of Adropin and Preptin Levels in the blood serum of *Cyprinion macrostomus* (Heckel, 1843)

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Abstract

Adropin and preptin have an important role in a range of physiological functions, from regulation of food intake to reproduction, immune function, energy expenditure, and lipid and carbohydrate metabolism. In this study, adropin and preptin levels were determined in the serum of *Cyprinion macrostomus*. The results were then compared based on gender. At the same time, the levels of these hormones were compared depending on length and weight. Hormone levels were measured by ELISA (Enzyme Labeled-Immunosorbent Analysis) method. It was determined that there was no significant difference between fish of different sexes in terms of adropin level in *C. macrostomus* ($p > 0.05$), but there was a statistically significant difference in terms of preptin level ($p < 0.05$). No correlation was found between serum adropin level of *C. macrostomus* and length ($r = 0.76$, $p = 0.06$) and weight ($r = 0.136$, $p = 0.916$).

Key words: Adropin, Preptin, *Cyprinion macrostomus*, ELISA, Metabolic hormone

INTRODUCTION

In recent years, many studies have been conducted on molecules that play a role in nutrient intake and energy balance. Although these molecules, preptin and adropin have been discovered before, they have not been studied much in fish (Lian et al., 2016; Caf et al., 2017; Zhang et al., 2020). Preptin is a 34 amino acid long peptide derived from the E-domain of a precursor of insulin-like growth factor 2 (pro-IGF2) with bone anabolic and insulin secretion enhancing properties (Lubos et al., 2022). Discovered in 2001, it is a peptide hormone associated with carbohydrate metabolism, predominantly synthesized in response to postprandial glucose from pancreatic beta cells (Cooper et al., 1987; Buchanan *et al*., 2001). Physiologically, it has been reported to mediate glucose concentration-dependent insulin secretion. This information indicates that preptin increases rather than initiates insulin secretion from beta cells in response to glucose (Buchanan et al., 2001). A positive relationship has been shown between preptin and insulin resistance in humans (Yang et al., 2009), indicating that preptin plays a role in the pathogenesis of insulin resistance without affecting insulin secretion (Yang et al., 2009). Studies have shown that plasma preptin levels are positively correlated with triglycerides, total cholesterol, and HbA1c (Yang, 2009). Adropin is a hormone synthesized in many peripheral tissues, especially liver and brain, which is encoded by the energy homeostasis-related gene (encho) (Kumar et al., 2008). It plays an important role in lipid metabolism, maintenance of insulin sensitivity and energy homeostasis. Systemic administration of adropin in diet-induced obese mice significantly reduces insulin resistance and glucose intolerance, which are key components in the metabolic stress response (Kumar et al., 2008). Adropin has been shown to reduce food intake and body weight when administered intraperitoneally in obese mice (Kumar et al., 2008). Since it is a factor regulating glucose and lipid metabolism, it has a protective effect against obesity-related hepatosteatosis and hyperinsulinemia (Kumar et al., 2008). Low adropin level in type 2 diabetes has been found to be associated with endothelial dysfunction (Topuz et al., 2013). Serum and tissue adropin levels are increased in rats with diabetes (Aydın et al., 2013). It is thought that adropine treatment rapidly decreases lipogenesis-related gene expression in the liver and causes a decrease in lipogenesis-related gene expression in liver and adipose tissues in transgenic mice with adropin overexpression (Kumar *et al*., 2008). While encho expression in the liver shows a negative relationship with lipogenic activity, it shows low expression with a high carbohydrate diet, and its expression increases with low carbohydrate and high fat consumption (Kumar et al., 2008). A negative correlation was observed between plasma adropin concentration and fasting triglyceride level (Butler et al., 2012). Dietary intake that is high in simple carbohydrates

together with adropin deficiency increases the risk of insulin resistance and metabolic syndrome development (Ganesh-Kumar et al., 2012). Adropin production is reduced as a secondary consequence of weight gain in the obese mouse model. Increased adropin level in the blood in response to metabolic stress reduces insulin resistance and glucose intolerance (Kumar et al., 2008).

Cyprinion macrostomus (Heckel, 1843), which belongs to the Cyprinidae family, is omnivorous, feeds on phytoplankton and zooplankton (Geldiay & Balık, 2007) and is a potential fish for human nutrition (Sen Özdemir et al., 2023). Its main distribution is India. This species, which covers the Asia Minor, Tigris and Euphrates river systems (Geldiay & Balık, 2007), lives in streams and shallow areas where streams mix with the dam lake (Yıldırım, 2012).

The main point in the selection of these hormones in the study; These hormones are closely related to the body's energy metabolism and have not been studied in this fish. It was investigated how these peptides, which play a role in energy conservation and nutrition, change depending on age, weight and sex in this fish.

MATERIALS AND METHOD

Working Area

This study is the longest of the two branches of the Euphrates in Eastern Anatolia, the 722 km long Murat River, which is formed by the merging of the branches originating from Aladağ and Muratbaşı Mountain in the north of Van Lake and flowing into the Keban Dam with a westward movement. It was carried out in the part of the river (Murat Water or Murat River) within the borders of Bingöl. The study was initiated with the approval of Bingöl University Animal Experiments Local Ethics Committee, dated 27.03.2015 and numbered 2015/02-01/02.

A total of 23 (male-female) fish were used in this study. Physical characteristics of fish are given in Table 1.

Table 1: Sex Determination and Weight and Length Measurements in Fish Used in the Study

No	Weight (g)	Length (cm)	Sex
1	60	16.5	Female
2	44	14.5	Male
3	33	13.5	Male
4	46	15	Male
5	54.5	16	Female
6	71	17.5	Female
7	50.5	15.5	Female
8	69	18	Male
9	59	17	Female
10	82.5	19	Female
11	47	16	Female
12	72.5	18	Female
13	60	16.5	Female
14	110	20	Female
15	64.5	17	Female
16	55	16	Female
17	67	17	Male
18	54.5	15.5	Male
19	56	16	Male
20	56.5	16.5	Female
21	56.5	17	Female
22	59	17	Female
23	59.5	17	Female

Collecting and Analysis of Blood Samples from Fish

After the fish were anesthetized with MS-222, blood samples were taken from the caudal vena. The height and weight of the fish were measured and recorded. Blood samples were taken into tubes containing aprotinin to prevent protein desaturation. Immediately after the blood samples were taken, they were centrifuged in a cooled centrifuge at +4°C, 4500 rpm for 5 minutes, and serum was obtained. Serums were stored at -80°C until analysis. Serum adropin (MyBioSource San Diego, California, USA. Catalog Number: MBS092568) and preptin (MyBioSource San Diego, California, USA. Catalog Number: MBS108945) levels were measured by ELISA using commercial kits. The operating procedure of these kits is the same, so they are provided together. All reagents and samples were brought to room temperature (25ºC) 30 minutes before the experiment started. Standard, samples and blank/control wells were prepared. 50 µl of standard 4, 3, 2, 1 and stock standard were added to the wells reserved for loading standards on the standard plate. One well was reserved for control. 50 µl of sample was loaded into the remaining wells. 100 µl of HRP-conjugate reagent was added to each well. The plate was closed with a plate sealer and incubated at 37°C for 60 minutes. The plate sealant

was removed and the contents of the wells emptied. The plate was washed 4 times with 350 µl of 1X wash solution and dried. This process is done automatically. 50 µl of Chromogen A and Chromogen B Solutions were added to each well. It was incubated for 15 minutes at 37°C with gentle mixing, protected from light. 50 µl of stop solution was added to each well and reading was made in the ELISA device at 450 nm within 15 minutes.

Values are expressed as means ± SEM. The Kolmogorov–Smirnov Z-test showed that the data were normally distributed. Pearson correlation analysis was used as a statistical method.

RESULT AND DISCUSSION

There are various studies on *C. macrostomus*. The natural living temperature and lethal temperature values of *C. macrostomus* (Al-Habbib & Al-Habbib, 1979), biological properties (Özer et al., 1987), the effect of nutritional fatty acids and starvation on the muscle tissue fatty acid composition of this fish (Akpınar, 1999), intestinal parasites (Saygı & Bardakçı, 1990), the effect of high temperature on this fish (Undar et al., 1990), some heavy metals (Gümgüm et al., 1994), hematological parameters and determination of innate immune response (Örün et al., 2003; Duman & Şahan, 2011) The seasonal variation of total lipid and fatty acid amount in gonads (Metin & Akpınar, 2000), the partial purification of small intestine lipase and the effect of pH on enzyme activity (Metin & Akpınar, 2000), the amount of calcium in the bone structure used for age determination (Aydın et al., 2008), as a part of health tourism, studies such as the treatment of psoriasis with doctor fish (Sayılı et al., 2007). However, no research has been found on metabolism-regulating hormones in this fish.

In the study, the physical characteristics (weight, length, sex) of *C. macrostomus* caught from the part of the Murat River within the borders of Bingöl Province were determined. By looking at the serum levels of preptin and adropin hormones, their changes according to these physical parameters were revealed. The mean length and weight of *C. macrostomus* were calculated as (16.6±1.4 cm and 60.32±3.14 g).

Adropin levels of males and females of *C. macrostomus* were determined as 121.29±8.73 pg/mL and 131.78±4.5 pg/mL, respectively (Figure 1). It was determined that there was no statistically significant difference in adropin level between fish of different sexes of *C. macrostomus* ($p > 0.05$). No correlation was found between serum adropin level of *C. macrostomus* and length ($r = 0.76$, $p = 0.06$) and weight ($r = 0.136$, $p = 0.916$). Although recent studies have shed light on the role of adropin on feeding behavior, what is known about it is limited. In the studies conducted by the team who discovered the hormone adropin, it was

determined that the level of adropin decreased in mice with food restriction, and the level of adropin increased in mice without food restriction.

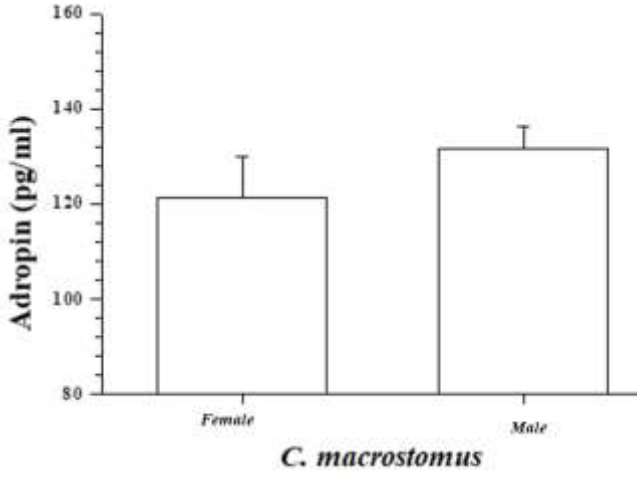

Figure 1: Adropin levels of males and females for *C. macrostomus* ($p < 0.05$).

Preptin levels of males and females of *C. macrostomus* were determined as 12.9±2.07 ng/mL and 6.04±1.6 ng/mL, respectively (Figure 2). It was determined that there was a statistically significant difference in terms of preptin level between fish of different sexes of *C. macrostomus* ($p < 0.05$). It was determined that there was a positive correlation between serum preptin level of *C. macrostomus* and length ($r = 0.649$, $p = 0.02$) and weight ($r = 0.640$, $p = 0.002$). In a study conducted with Wister rats, the preptin level in females was found to be higher than in males (Buchanan *et al*., 2001). Serum levels of adropin and preptin hormones have been studied in *Alburnus tarichi* and it has been reported that serum levels of these hormones are independent of sex, body weight and length (Caf et al., 2017).

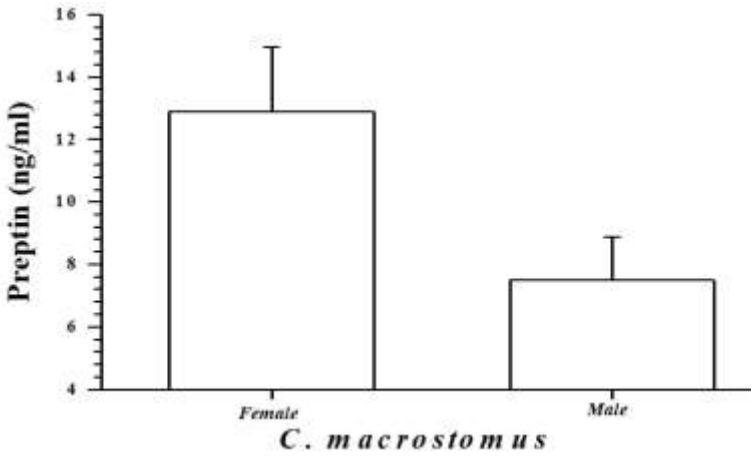

Figure 2. Preptin levels of males and females of *C. macrostomus* ($p < 0.05$).

The peptide hormone adropin has important roles in energy homeostasis. However, the biological functions of adropin in non-mammalian species are still unknown. In a study using tilapia as a model, the role of adropin in the regulation of lipoprotein lipase (LPL) in hepatocytes was investigated. In a study using tilapia as a model, the role of adropin in the regulation of lipoprotein lipase (LPL) in hepatocytes was investigated. In tilapia, adropin is present in various tissues and its high levels are expressed in the liver and hypothalamus (Lian et al., 2016). In another study that tried to determine the roles and underlying mechanisms of adropin in glucose and lipid metabolism in Nile tilapia; It has been emphasized that it can suppress hepatic gluconeogenesis and triglyceride accumulation through a mechanism dependent on AMPK signaling (Zhang et al., 2020). Adropin has been detected in many organs, especially in the hypothalamic areas where food intake and body weight are regulated, and in liver and muscle tissue (Aydın et al., 2013). It was detected in fish serum in this study. It has been reported that there are two isoforms of adropine in Nile tilapia and these forms have different biological effects. It was emphasized that while adropin-b could promote food intake, adropin-a had no effect in this regard (Zhang et al., 2021). As a result of the study, the difference in hormone values in males and females supports the potential metabolic roles of these hormones in fish. It has been suggested that adropin and preptin play an important role in a number of physiological functions in humans and other mammalian groups, including regulation of food intake, reproduction, immune function, energy expenditure, and lipid and carbohydrate metabolism. We think that this study is a step in determining the functions of these hormones in fish and will shed light on more comprehensive studies.

As a result, it was determined that there was no significant difference between fish of different sexes in terms of adropin level in *C. macrostomus* ($p > 0.05$), but there was a statistically significant difference in terms of preptin level ($p < 0.05$). No correlation was found between serum adropin level of *C. macrostomus* and length ($r = 0.76$, $p = 0.06$) and weight ($r = 0.136$, $p = 0.916$). It was determined that there was a positive correlation between serum preptin level of *C. macrostomus* and length ($r = 0.649$, $p = 0.02$) and weight ($r = 0.640$, $p = 0.002$).

Acknowledgement

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Conflict of Interest

There is no conflict of interest

Animal Welfare Statement

Appropriate ethical review approval was received from the Animal Experiments Local Ethics Board of Bingöl University.

Authorship Statement

FC and EB did experimental design and article writing.

SA was involved in experiment design and data analysis

Data Availability

Data supporting the findings of this study can be obtained from the corresponding author if deemed necessary.

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