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RESEARCH PAPER

The Correlation between the Differences in NUCB2/Nesfatin(NES) Peptide Levels and Body Weight, Lenght and Gender in *Alburnus tarichi*

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Abstract

Nucleobindin-2 (NUCB2) is a 396-amino-acid peptide related to food intake and body weight. It suppresses appetite by acting on the hypothalamus, andits derived peptide, nesfatin-1, was shown to reduce food intake and body weight in rodents. In this study, we examined NUCB2 peptide levels in correlation with body weight, length and gender in adult *Alburnus tarichi* fish. Serum NUCB2 levels were measured with ELISA, and were observed to significantly vary depending on gender:0.43±0.17 ng/mL in the males (n=6) and 0.28±0.11 ng/ml in the females (n=6) ($p<0.05$). However, NUCB2 levels were not significantly correlated to body weight ($r=-0.08$, $p=0.7$) or length ($r=-0.16$, $p=0.6$).NUCB2/nesfatin, an appetite-regulation hormone, was therefore shown to be impacted by gender but not by body weight or length in *A. tarichi*.

Keywords: NUCB2/nesfatin, *Alburnus tarichi*, body weight and length, gender, ELISA.

Introduction

Various stimulants released from peripheral tissues such as the brain, gastric fat, pancreas and gastrointestinal tract have been discovered in recent years and can affect various regions of the hypothalamus as well as the blood serum (Köprücü & Algül, 2015; Unniappan &Peter, 2005; Volkoff, 2005). One of these new stimulants is the hormone NUCB2/nesfatin, which is released by the hypothalamus. The NUCB2/nesfatin protein contains 396 amino acids and carries a 24-amino acid precursor signal. NUCB2 encodes three types of nesfatin proteins: nesfatin-1(1–82), nesfatin-2(85–163) and nesfatin-3(166–396). Nesfatin-1 is an anorexigenic hormone that inhibits food intake and body weight in mammals; central injections of this hormone caused reduced food intake and body weight in rats (Maejima et al., 2009; Oh-I et al., 2006; Shimizu et al., 2009; Stengel et al., 2009).Functional analysis of the nesfatin-1 protein, which has been shown to have three functional regions, revealed the anorexia-causing region to be in the middle (Shimizu et al., 2009).Recently, several researchers reported similar findings in fish (Gonzalez, Kerbel, Chun, & Unniappan, 2010; Hatef, Shajan, & Unniappan, 2015; Lin et al., 2014). However, nesfatin-2 and nesfatin-3 had no anorexic effects (Garcia-Galiano, Navarro, Gaytan, & Tena-Sempere 2010).

Alburnus tarichi, a member of the Cyprinidae family, lives only in the Lake Van Basin in Turkey. Lake Van is an interesting ecosystem that is highly alkaline (pH 9.8) and known as the largest soda lake in the world (Sarı, 2008). Its saline and alkaline waters do not support animal life except for the *A. tarichi* fish (Arabaci & Sari, 2004; Kempe et al., 1991). *A. tarichi* is an important species, with an average annual inland fish production of 10,000 tons, a significant proportion of the totalof 40–45 thousand tons in Turkey (Elp, Şen, & Atıcı, 2014).In this study, we aimed to determine whether NUCB2/nesfatin concentrations in the serum of *A. tarichi* were correlated with differences in body weight, length and gender. This study provides more information about the influence of NUCB2 on food intake in fish, which will be useful in the future biotechnology purposes for improvement of the aquaculture production.

Materials and Methods

Twelve adult *A. tarichi* (6 females and 6 males) were obtained from Van Lake (Karasu River, Van, Turkey). The Karasu is one of the sites of *A. tarichi*'s intensive reproductive migration. According to the procedures approved by the Animal Ethics Committee Van University, the fish were anaesthetized with 100

ppm tricainemethane-sulfonate, then gender discrimination was performed. The length (cm) and weight (g) of each *A. tarichi* were measured, and blood samples were taken from caudal veins. The samples were collected in tubes containing aprotinin to prevent desaturation of the proteins, then centrifuged after the completion of clot formation and stored at −80°C until the day of the hormone measurements (Hettich, Zentrifugen Universal 32 R, Germany). NUCB2/nesfatin was determined with ELISA kits to have a sensitivity of 0.1 ng/mL (Fish nesfatin(NES) ELISA Kit, Cat. No: MBS013992). The plates were then read at 450 nm with a Spectra Max Plus384 plate reader (Molecular Devices LLC, Sunnyvale, CA, USA).

Values are expressed as mean±SD. 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 differences between the sexes of each species. A p-value of <0.05 was accepted as statistically significant, and p<0.0001 was accepted as highly statistically significant.

Results

Individual NUCB2/nesfatin values for each fish are shown in Figure 1.The mean length and weight values for the *A. tarichi* were 22.12±1.4 cm and 88.63±14.3 g, respectively (Fig.1).

The NUCB2/nesfatin levels in the 6 males and 6 females were statistically significantly different (0.43±0.17 ng/ml versus 0.28±0.11 ng/ml, respectively; p<0.05) (Figure 2). There was no significant correlation between NUCB2/nesfatin levels and body weight (r=−0.08, p=0.7) or length (r=−0.16, p=0.6).

The physical properties of the water were measured using a multi-parameter probe (Temperature 22.2 °C, salinity 0.29 %, pH 8.75, dissolved oxygen 7.99).

Discussion

Nucleobindins are important regulators of various cellular functions. The discovery of NUCB2/nesfatin-1 led to the identification of a prominent new role for nucleobindins, which are precursors of endocrine factors. The biological roles of NUCB2/nesfatin-2 and NUCB2/nesfatin-3 are still unknown (Gonzalez et al., 2012).

To date, limited studies have focused on NUCB2/nesfatin in fish (Blanco, Bertucci, Delgado, Valenciano, & Unniappan,2016; Gonzalez, Kerbel,

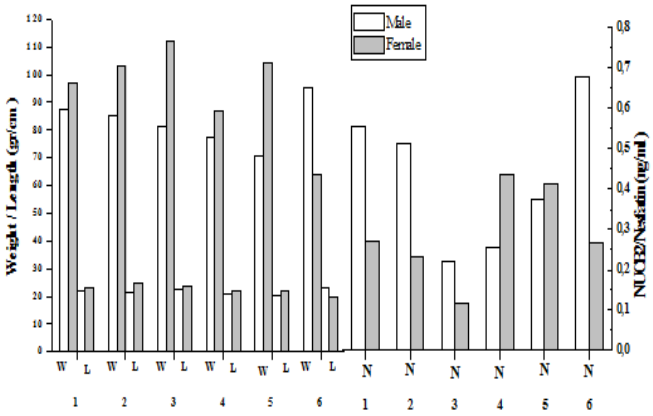

Figure 1. Individual NUCB2/nesfatin variation values and individual weight, length variation values of *A. tarichi* (N: Nesfatin, W: weight, L: lenght

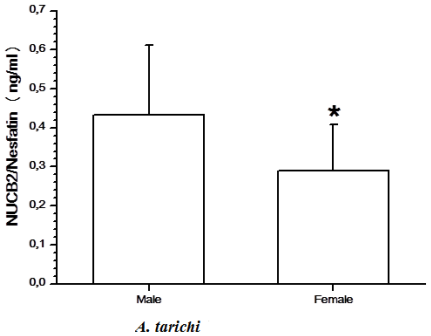

Figure2. Serum NUCB2/nesfatin levels of 6 male and 6 female *A. tarichi* (mean±SD, P<0.05).

Chun, & Unniappan, 2010; Hatef, Shajan, & Unniappan, 2015;Kerbel &Unniappan, 2012).Studies have shown that the NUCB2/nesfatin peptide and its product, nesfatin-1, are expressed in the hypothalamus, peripheral tissues (including adipose tissue), brain, stomach, kidney, nucleus lateralis tuberis, anterior pituitary, gonads, gastrointestinal tract and follicular cells in fish (Gonzalez, Kerbel, Chun, & Unniappan, 2010; Gonzalez et al., 2012; Hatef, Shajan, & Unniappan, 2015; Kerbel &Unniappan, 2011; Lin et al., 2014). In the present study, NUCB-2/nesfatin was identified in the blood serum of *A. tarichi*.

There are two isoforms of NUCB2: NUCB2A and NUCB2B.In zebra fish, NUCB2A and NUCB2 mRNA is expressed mostly in the liver and less in other tissues, including the brain and intestine (Hatef, Shajan, & Unniappan, 2015). The presence of NUCB2 in tissues at different ratios can be attributed to its potential metabolic roles. NUCB2 mRNA expression has also been detected in the brain, hepatopancreas, adipose tissue, intestine, ovary and liver of *Carassius auratus* fish (Gonzalez, Kerbel, Chun, & Unniappan, 2010) and *Schizothorax prenanti* (Lin et al., 2014). The study by Gonzalez, Kerbel, Chun, and Unniappan (2010) was done to determine the effect of feeding on nesfatin-1 levels in *C. auratus* and found that the fish deprived of food had lower serum nesfatin-1 levels compared to regular-diet controls. Nesfatin-1 also remained significantly increased in the circulation (5.97±2.19 ng/ml) until 1 hour after feeding.

In this study, NUCB2/nesfatin was determined with ELISA, a quantitative analytical method that demonstrates antigen-antibody reactions via colour changes based on an enzyme-linked conjugate and enzyme substrate, and determines the presence and concentration of molecules in biological fluids (Aydın, 2015). In many studies on fish, PCR was used to measure NUCB2. NUCB2 mRNA expression has been determined in *Carassius auratus* (Gonzalez, Kerbel, Chun, & Unniappan, 2010; Gonzalez et al.,2012; Kerbel & Unniappan, 2012), *Oncorhynchus mykiss* (Caldwell, Pierce, Riley, Duncan, & Nagler, 2014), *Schizothorax prenanti* (Lin et al., 2014) and *Danio rerio* (Gonzales et al., 2012; Hatef, Shajan, & Unniappan, 2015).

However, protein expression does not reflect the exact circulating levels of NUCB2/nesfatin. Identification of peptide/protein levels in circulation is performed with the ELISA method because it is economical, can be used for measurements even at low concentrations, and provides quick results (İnci & Ünübol Aypak, 2016).

The 2006 finding that levels of nucleobindin-2 and its product, nesfatin-1, varied in rats according to body weight was of great interest (Oh-I et al., 2006). However, the relationship of NUCB2/nesfatin to body weight, length and gender had not previously been investigated in fish. The present study examined differences in NUCB2 peptide levels with regard to weight, length and gender in the fish species *A. tarichi*, and found no significant correlations between serum NUCB2/nesfatin and body weight ($r=-0.08$, $p=0.7$) or length ($r=-0.16$, $p=0.6$). However, NUCB2/nesfatin levels were significantly different between the genders in *A. tarichi*.

References

Arabaci, M.,& Sari, M. (2004). Induction of ovulation in endemic pearl mullet (*Chalcalburnus tarichi*), living in the highly alkaline Lake Van, using GnRHa([DSer(tBu)6, Pro9-Net]-GnRH) combined with haloperidol. *Aquaculture*, 238, 529–535. http://dx.doi.org/ 10.1016/j.aquaculture.2004.05.004

Aydın, S. (2015). A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA.*Peptides*, 72, 4-15.http://dx.doi.org/10.1016/j.peptides.2015.04.012

Blanco, A.M., Bertucci, J.I., Delgado, M.J., Valenciano, A.I., & Unniappan S. (2016). Tissue-specific expression of ghrelinergic and NUCB2/nesfatin-1 systems in goldfish (Carassius auratus) is modulated by macronutrient composition of diets.*Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology*, 195, 1–9. http://dx.doi.org/ 10.1016/j.cbpa.2016.01.016

Caldwell, L.K., Pierce, A.L., Riley, A.G., Duncan, C.A., & Nagler, J.J. (2014). Plasma nesfatin-1 is not affected by long-term food restriction and does not predict rematuration among iteroparous female rainbow trout (*Oncorhynchus mykiss).PLoS ONE*, 9, 85700. http://dx.doi.org/10.1371/journal.pone.0085700

Elp, M., Şen, F., & Atıcı, A.A. (2014). İnci Kefalinin *(Alburnus tarichi* (Guldenstaedtii, 1814)) Van Gölü Havzası Su Kaynaklarındaki Yayılım Bölgeleri. *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi*, 24(3), 228-232.

Garcia-Galiano, D., Navarro, V.M., Gaytan, F., & Tena-Sempere M. (2010). Expanding roles of NUCB2/nesfatin-1 in neuroendocrine regulation. *Journal of Molecular Endocrinology*,45, 281–290. http://dx.doi.org/10.1677/JME-10-0059

Gonzalez, R., Kerbel, B., Chun, A., & Unniappan S. (2010). Molecular, cellular and physiological evidences for the anorexigenic actions of nesfatin-1 in goldfish. *PLoS ONE*, 5, 15201. http://dx.doi.org/10.1371/journal.pone.0015201

Gonzalez, R., Shepperd, E., Thiruppugazh, V., Lohan, S., Grey, C.L., Chang, J.P., & Unniappan, S. (2012). Nesfatin-1 regulates the hypothalamo-pituitary-ovarian axis of fish. *Biology of Reproduction*, 87, 1-11. http://dx.doi.org/ 10.1095/biolreprod.112.099630

Hatef, A.,Shajan, S., &Unniappan S. (2015). Nutrient status modulates the expression of nesfatin-1 encoding nucleobindin 2A and 2B mRNAs in zebrafish gut, liver and brain. *General and Comparative Endocrinology*,215, 51-60.http://dx.doi.org/10.1016/j.ygcen.2014.09.009

İnci, A.,& Ünübol Aypak, S. (2016). İrisin ve Metabolik Etkileri. *Turkiye Klinikleri Journal of Endocrinology*, 11(1), 15-21.https://doi.org/10.5336/endocrin.2016-49995

Kempe, S., Kazmierczak, J., Landmann, G., Konuk, T.,

Reimer, A., & Lipp, A. (1991). Largest known microbialites discovered in Lake Van, Turkey. *Nature*, 349, 605–608. http://dx.doi.org/10.1038/349605a0

Kerbel, B.,& Unniappan, S. (2012). Nesfatin-1 suppresses energy intake, co localises ghrelin in the brain and gut and alters ghrelin, cholecystokinin and orexin mRNA expression in gold fish. *Journal of Neuroendocrinology*,24, 366-377. http://dx.doi.org/10.1111/j.1365 2826.2011.02246.x

Köprücü, S., &Algül, S. (2015). Investigation of the leptin levels in the blood serum of *Cyprinus carpio* (Linnaeus, 1758) and *Capoeta trutta* (Heckel, 1843). *Journal of Animal Physiology and Animal Nutrition*, 99, 430-435. https://doi.org/10.1111/jpn.12271

Lin, F., Zhou, C., Chen, H., Wu, H., Xin, Z., Liu, J., Gao, Y., Yuan, D., Wang, T., Wei, R., Chen, D., Yang, S., Wang, Y., Pu, Y., & Li, Z. (2014). Molecular characterization, tissue distribution and feeding related changes of NUCB2A/nesfatin-1 in Ya-fish (*Schizothorax prenanti).Gene*,536, 238-246. http://dx.doi.org/10.1016/j.gene.2013.12.031

Maejima, Y., Sedbazar, U., Suyama, S., Kohno, D., Onaka, T., Takano, E., Yoshida, N., Koike, M., Uchiyama, Y., Fujiwara, K., Yashiro,T., Horvath, T.L., Dietrich, M.O., Tanaka, S., Dezaki, K., Oh-I, S., Hashimoto, K., Shimizu, H., Nakata, M., Mori, M., & Yada, T. (2009). Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. *Cell Metabolism*, 10, 355–365. https://doi.org/10.1016/j.cmet.2009.09.002

Oh-I, S., Shimizu, H., Satoh, T., Okada, S., Adachi, S., Inoue, K., Eguchi, H., Yamamoto, M., Imaki, T., Hashimoto, K., Tsuchiya, T., Monden, T., Horiguchi, K., Yamada, M., & Mori, M. (2006). Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature*, 443, 709–712. https://doi.org/10.1038/nature05162

Sarı, M. (2008). Threatened fishes of the world: *Chalcalburnus tarichi* (Pallas 1811) (Cyprinidae) living in the highly alkaline Lake Van, Turkey.*Environmental Biology of Fishes*, 81, 21–23.http://dx.doi.org/10.1007/s10641-006-9154-9

Shimizu, H., Oh-I, S., Hashimoto, K., Nakat,a M., Yamamoto, S., Yoshida, N., Eguchi, H., Kato, I., Inoue, K., Satoh, T., Okada, S., Yamada, M., Yada T., & Mori M.(2009). Peripheral administration of nesfatin-1 reduces food intake in mice: the leptin independent mechanism. *Endocrinology*, 150, 662–671. http://dx.doi.org/10.1210/en.2008-0598

Stengel, A., Goebel, M., Yakubov, I., Wang, L., Witcher, D., Coskun, T., Taché, Y., Sachs, G., & Lambrecht, N.W. (2009). Identification and characterization of nesfatin-1 immunoreactivity in endocrine cell types of the rat gastric oxyntic mucosa. *Endocrinology*, 150, 232–238.https://doi.org/10.1210/en.2008-0747

Unniappan S.,&Peter R.E. (2005). Structure, distribution and physiological functions of ghrelin in fish. *Comparative Biochemistry and Physiology, Part A*, 140, 396–408. https://doi.org/10.1016/j.cbpb.2005.02.011

Volkoff, H., Canosa, L.F., Unniappan, S., Cerda-Reverter, J.M., Bernier, N.J., Kelly, S.P., &Peter, R.E. (2005). Neuropeptides and the control of food intake in fish. *General and Comparative Endocrinology*, 142, 3–19. http://dx.doi.org/10.1016/j.ygcen.2004.11.001