

## Mini review:

### NESFATIN-1: ROLE AS POSSIBLE NEW ANTI-OBESITY TREATMENT

Carmine Finelli<sup>1</sup>, Giuseppe Martelli<sup>2</sup>, Rocco Rossano<sup>2</sup>, Maria Carmela Padula<sup>2</sup>,  
Nicolina La Sala<sup>1</sup>, Luigi Sommella<sup>3</sup>, Giovanni Tarantino<sup>4</sup>

<sup>1</sup> Center of Obesity and Eating Disorders, Stella Maris Mediterraneo Foundation,  
Chiaromonte, Potenza, Italy

<sup>2</sup> Department of Science, University of Basilicata, Potenza, Viale dell'Ateneo Lucano, 10,  
85100, Italy

<sup>3</sup> Unit of Surgery, S. Giovanni Hospital - Lagonegro, Potenza, Italy

<sup>4</sup> National Cancer Institute "Pascale Foundation" - IRCS- 83013 Mercogliano (Av), Italy

\* Corresponding author: Carmine Finelli, Center of Obesity and Eating Disorders,  
Stella Maris Mediterraneo Foundation, Chiaromonte, Potenza, Italy –  
e-mail: carminefinelli74@yahoo.it

#### ABSTRACT

In this article, we review on the current concepts about Nesfatin-1 as a new anti-obesity treatment and evaluate the existing issues in the context of this knowledge and the available literature. The intent is to enable clinicians to know Nesfatin-1 as a new anti-obesity treatment and make rational decisions based on this perspective as possible clinical application. Future research should seek to clarify whether Nesfatin-1 would be beneficial in the management of obesity.

**Keywords:** nesfatin-1, obesity, drug treatment

#### INTRODUCTION

Nesfatin-1 is an 82-amino-acid peptide originated from post translational processing of the N-terminal fragment of nucleobindin 2 (NUCB2), a 396-amino-acid protein exceptionally conserved across mammalian species (Oh-I et al., 2006). Post translational cleavage of NUCB-2 appears in the expression of nesfatin-2 fragment (85–163) and nesfatin-3 fragment (166–396) in addition to nesfatin-1 (Oh-I et al., 2006). Oh-I et al. (2006) suggested that nesfatin-1 (named as acronym for NEFA/nucleobindin2-encoded satiety- and fat-influencing protein) may have physiological importance in regulating food intake.

In fact, nesfatin-1 injected into the third brain ventricle reduces food consumption occurring during the dark phase, whereas nesfatin-2 or nesfatin-3 had no effect (Oh-I et al., 2006). In the same way, continuous infusion of nesfatin-1 (5 pmol/day for 10 days into the third brain ventricle) decreases significantly food intake and body weight gain in rats (Oh-I et al., 2006). Conversely, third ventricle infusion of a NUCB-2 antisense oligonucleotide increases food intake and body weight gain compared with a missense NUCB-2 oligonucleotide (Oh-I et al., 2006). Additionally, a 24-h fast decreased the expression of NUCB-2 in the paraventricular nucleus (PVN) (Oh-I et al., 2006).

Some studies (Oh-I et al., 2006; Zheng et al., 2009; Ishida et al., 2012; Albayrak et al., 2013) showed high expression level of nesfatin-1/NUCB-2 expression in relevant hypothalamic and medullary sites implicated in feeding regulation in rats. The arcuate nucleus, PVN, and the nucleus of the solitary tract (NTS), further supporting the evidence that nesfatin-1 is involved in food intake regulation.

Nesfatin-1 is co-expressed with Melanin-Concentrating Hormone (MCH) in neurons from the tuberal hypothalamic area (THA) which are recruited during sleep states, especially paradoxical sleep (PS) (Jego et al., 2012). To help decipher the contribution of this contingent of THA neurons to sleep regulatory mechanisms, Jego et al. (2012) thus investigated in rats whether the co-factor Nesfatin-1 is also endowed with sleep-modulating properties. It was found that the disruption of the brain Nesfatin-1 signaling achieved by icv administration of Nesfatin-1 antiserum or antisense against the nucleobindin2 (NUCB2) prohormone suppressed PS with little, if any alteration of slow wave sleep (SWS) (Jego et al., 2012). Additionally, the infusion of Nesfatin-1 antiserum after a selective PS deprivation, designed for elevating PS needs, severely prevented the ensuing expected PS recovery (Jego et al., 2012). Strengthening these pharmacological data, Jego et al. (2012) finally demonstrated by using c-Fos as an index of neuronal activation that the recruitment of Nesfatin-1-immunoreactive neurons within THA is positively correlated to PS but not to SWS amounts experienced by rats prior to sacrifice. In conclusion, this work supports a functional contribution of the Nesfatin-1 signaling, operated by THA neurons, to PS regulatory mechanisms. Jego et al. (2012) proposed that these neurons, likely releasing MCH as a synergistic factor, constitute an appropriate lever by which the hypothalamus may integrate endogenous signals to adapt the ultradian rhythm and maintenance of PS in a manner dictated by homeostatic needs. This could be done through the inhibition of

downstream targets comprised primarily of the local hypothalamic wake-active orexin- and histamine-containing neurons (Jego et al., 2012).

Activation of brain CRF signaling pathways by CRF acting on CRF1 and CRF2 receptors and by selective endogenous CRF2 agonists urocortin 2 or 3 (Chen et al., 2013) inhibits food intake (Doyon et al., 2004). Nesfatin-1 injected intracerebroventricularly significantly decreased gastric emptying (García-Galiano et al., 2012). Goebel-Stengel and Wang (2012) showed that NUCB2/nesfatin-1 immunoreactivity is distributed in mouse brain areas involved in the regulation of stress response and visceral functions activated by an acute psychological stressor suggesting that nesfatin-1 might play a role in the efferent component of the stress response.

#### **NESFATIN-1/NUCB-2 AND ANOREXIGENIC EFFECT**

Peptides regulating food intake often act in concert or in series with other neurotransmitters to exert their actions (Perry and Wang, 2012). Nesfatin-1/NUCB-2 is colocalized with a number of hypothalamic peptides regulating food intake (Fort et al., 2008; Shimizu et al., 2009b; Maejima et al., 2009; Yoshida et al., 2010; Tan et al., 2011; Kerbel and Unniappan, 2012; Goebel-Stengel and Wang, 2013). Several interactions have been described to underlie the central anorexic effect of nesfatin-1 (Shimizu et al., 2009c). It has also been shown to play important roles in the control of cardiovascular function (Mimee et al., 2012). In situ hybridization and immunohistochemical researches have evidenced the expression of nesfatin-1 throughout the brain and, particularly, in the medullary autonomic gateway known as the nucleus of the solitary tract (NTS) (Mimee et al., 2012). Mimee et al. (2012) showed that provide critical insight into the circuitry and physiology involved in the profound effects of nesfatin-1 and highlight the NTS as a key structure mediating these autonomic actions.

Two proteins have been localized in the arcuate nucleus (ARC) and implicated in the regulation of food intake: the serine-threonine-kinase mammalian target of rapamycin (mTOR) as part of the TOR signaling complex 1 (TORC1), and nesfatin-1 derived from the precursor protein nucleobindin2, as reported by Inhoff et al. (2010). In fact, nesfatin-1 is not only intracellularly co-localized with cocaine- and amphetamine-regulated transcript (CART) peptide as reported before, but also with phosphomTOR (pmTOR) and neuropeptide Y (NPY) in ARC neurons (Inhoff et al., 2010). This data could also confirm results from previous studies, showing that the majority of nesfatin-1 neurons are also positive for CART peptide, whereas most of the pmTOR is co-localized with NPY and only to a lesser extent with CART (Inhoff et al., 2010).

A study, described by Maejima et al. (2009), provided a strong evidence for the involvement of oxytocin pathway in nesfatin-1's inhibitory effect on food intake. First of all, oxytocin injected into the 3v reduces food intake *via* a leptin-independent mechanism (Maejima et al., 2009). At the same time nesfatin-1 injected into the 3v activates oxytocin-positive neurons in the magnocellular part of the PVN as assessed by double labelling for Fos/oxytocin immunoreactivity and *in vitro* it stimulates the release of oxytocin from PVN neurons (Maejima et al., 2009). In addition there is pharmacologic and anatomical support for oxytocinergic projections from the PVN to the nucleus of the solitary tract to be involved in the anorexigenic signalling of nesfatin-1 (Maejima et al., 2009). An oxytocin receptor antagonist injected into the hindbrain at the level of the 4v blocked the food intake reducing effect of nesfatin-1 injected into the PVN and tracing studies showed synaptic contacts between oxytocinergic nerve terminals and proopiomelanocortin (POMC) neurons in the nucleus of the solitary tract. Yosten and Samson (2010) also showed that an oxytocin antagonist injected intracerebroventricular blocks the food intake suppressing effects of

intracerebroventricular nesfatin-1 and  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH). Therefore, nesfatin-1 acts through a serial neuronal circuit, in which nesfatin-1 activates the central melanocortin system, which, in turn, acts through the central oxytocin system, leading to an inhibition of food and water intake and an increase in mean arterial pressure (Yosten and Samson, 2010).

Future research should seek to clarify whether the hypothalamic/nesfatin-1-oxytocin-brainstem/POMC signalling is the predominant pathway or an intrahypothalamic nesfatin-1-POMC/oxytocin network exists.

Some studies showed that based on the observation of a delayed and long lasting anorexigenic effect, following intracerebroventricular injection of nesfatin-1 which mimics the characteristics of the food intake reducing effect of CRF2 receptor agonists, urocortins (Chen et al., 2013; Yakabi et al., 2011). The possible involvement of the CRF2 receptors in the mediation of nesfatin-1's effect was investigated. The CRF2 antagonist, astressin2-B (Wang et al., 2013), injected intracerebroventricular completely abolished the dark phase food intake reduction induced by intracerebroventricular nesfatin-1 (Stengel et al., 2009). By contrast, a control peptide of similar structure as astressin2-B but without affinity to the CRF2 receptor did not influence intracerebroventricular nesfatin-1's action (Stengel et al., 2009). However as reported by Stengel et al. (2009), astressin2-B injected intracerebroventricular did not modulate the rapid onset reduction of food intake observed after intracerebroventricular injection of nesfatin-1. In contrast to the effect on food intake, the CRF2 antagonist, astressin2-B injected intracerebroventricular did not alter the intracerebroventricular nesfatin-1 induced delayed gastric emptying (Stengel et al., 2009) giving rise to different downstream signalling pathways mediating intracerebroventricular nesfatin-1's inhibitory effects on food intake and gastric transit. Finally, as reported by Yosten and Samson (2010), the melanocortin 3/4 receptor antagonist, SHU9119 injected

intracerebroventricular diminished, and into the 3v abolished (Oh-I et al., 2006), the anorexigenic effect of nesfatin-1. Nesfatin-1 probably act in series through the recruitment of the central melanocortin and CRF2's pathways to reduce food intake.

Another, intracerebroventricular administration of nesfatin-1 induced c-Fos expression in CRF neurons, and nesfatin-1 increased cytosolic Ca<sup>2+</sup> concentrations in single CRF neurons in the PVN (Jia et al., 2013). It is now well established that the brain CRF/CRF1 signaling system modulates pain responses (Jia et al., 2013). These observations suggest that nesfatin-1 may be involved in the autonomic regulation of visceral sensation (Jia et al., 2013). Jia et al. (2013) suggested that nesfatin-1 may be associated with the visceral hypersensitivity state of irritable bowel syndrome, and this may be mediated, at least in part, by brain CRF/CRF1 signaling pathways.

#### **NESFATIN-1 AND ANTI-OBESITY TREATMENT**

It has also been postulated that nesfatin-1/NUCB-2 may be produced by the hypothalamus (Goebel-Stengel and Wang, 2013); the relatively high CerebroSpinalFluid (CSF)/plasma nesfatin-1/NUCB-2 ratios suggest that a substantial amount of CSF nesfatin-1/NUCB-2 may originate from central neurons. The possible discrepancy in the production of nesfatin-1/NUCB-2, by these central neurons may account for the differences in CSF/plasma nesfatin-1/NUCB-2 ratio between obese and lean subjects observed by Tan et al. (2011). Furthermore, it is probable that nesfatin-1/NUCB-2 has protein binding, and that differences in protein binding in obese and lean subjects may also explain these findings. Finally, it is possible that the efficiency of nesfatin-1/NUCB-2 uptake into CSF is reduced in obese individuals, possibly due to saturation of transporters (Tan et al., 2011). Further studies could be useful to elucidate this hypothesis. Some data demonstrated that nesfatin-1/NUCB-2 is a novel depot-specific adipokine preferentially

expressed in subcutaneous adipose tissue/adipocytes. Adipose tissue nesfatin-1/NUCB-2 expression increases with obesity and is altered in states of feeding and malnutrition (Ramanjaneya et al., 2010).

Reduced leptin sensitivity or leptin resistance is a common phenomenon in obesity (Ramanjaneya et al., 2010). In the light of this and the pre-clinical findings that central and peripheral injection of nesfatin-1 exerts its food reducing effects *via* a leptin-independent mechanism (Oh-I et al., 2006; Shimizu et al., 2009a), targeting nesfatin-1 may be a promising approach in the drug treatment of obesity and its complications. Ongoing pre-clinical data suggest the possible use of subcutaneous and intranasal routes of nesfatin-1 administration (Shimizu et al., 2009c), which needs to be further explored. Another important aspect to be unraveled is the weight loss upon chronic administration of nesfatin-1 (Li et al., 2010; Gonzalez et al., 2012; Li et al., 2013; Dong et al., 2013) and possible related changes in energy balance and/or basal metabolic rate (Foo et al., 2008; García-Galiano et al., 2010; Gonzales et al., 2011; Pałasz et al., 2012; Kerbel and Unniappan, 2012; García-Galiano et al., 2012; Ghanbari Niaki et al., 2013; Vas et al., 2013) for which studies so far are limited. In fact, nesfatin-1 has a remarkably prolonged effect on food intake and body temperature (Könczöl et al., 2012). Time course of nesfatin-1's effects may be varied depending on the time applied (Könczöl et al., 2012). Many of the nesfatin-1/NUCB2 neurones are cold sensitive, and are positioned in key centres of thermoregulation (Könczöl et al., 2012). Nesfatin-1 regulates energy expenditure a far more potent way than it was recognised before making it a preferable candidate anti-obesity drug (Könczöl et al., 2012).

Future research should seek to clarify whether nesfatin-1/NUCB-2 would be beneficial in the management of obesity. Another, bariatric surgery produces differential influences with regards to circulating nesfatin-1 and in particular the receptor involved in the peptide's actions, and the processing of

NUCB2 to nesfatin-1 in hypothalamic or gut tissues still remain elusive (Finelli et al., 2014).

### CONCLUSIVE REMARKS

The data obtained in basic experiments of nesfatin-1 should be useful for the development of a new anti-obesity treatment. However, the mechanisms of endocrine and metabolic effects of nesfatin-1 have not been known well by now, and the influences of nesfatin-1 administered peripherally should be much more clarified *in vivo* before starting controlled clinical trials in the future. In fact, the details of nesfatin-1 physiology ought to be clarified, and it may be considered suitable in the future, as a potential drug in the pharmacotherapy of obesity. On the other hand, some putative nesfatin-1 antagonists may improve eating disorders (Pałasz et al., 2012)

### DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

### REFERENCES

- Albayrak A, Demiryilmaz I, Albayrak Y, Aylu B, Ozogul B, Cerrah S et al. The role of diminishing appetite and serum nesfatin-1 level in patients with burn wound infection. *Iran Red Crescent Med J* 2013;15:389-92.
- Chen P, Hover CV, Lindberg D, Li C. Central urocortin 3 and type 2 corticotropin-releasing factor receptor in the regulation of energy homeostasis: critical involvement of the ventromedial hypothalamus. *Front Endocrinol* 2013;3:180.
- Dong J, Xu H, Xu H, Wang PF, Cai GJ, Song HF et al. Nesfatin-1 stimulates fatty-acid oxidation by activating AMP-activated protein kinase in STZ-induced type 2 diabetic mice. *PLoS One* 2013;8:e83397.
- Doyon C, Moraru A, Richard D. The corticotropin-releasing factor system as a potential target for anti-obesity drugs. *Drug News Perspect* 2004;17:505-17.
- Finelli C, Padula MC, Martelli G, Tarantino G. Could the improvement of obesity-related co-morbidities, following bariatric surgery, depend on modified gut hormones secretion? *World J Gastroenterol* 2014, in press.
- Foo KS, Brismar H, Broberger C. Distribution and neuropeptide coexistence of nucleobindin-2 mRNA/nesfatin-like immunoreactivity in the rat CNS. *Neuroscience* 2008;156:563-79.
- Fort P, Salvart D, Hanriot L, Jegou S, Shimizu H, Hashimoto K et al. The satiety molecule nesfatin-1 is co-expressed with melanin concentrating hormone in tuberal hypothalamic neurons of the rat. *Neuroscience* 2008;155:174-81.
- García-Galiano D, Navarro VM, Roa J, Ruiz-Pino F, Sánchez-Garrido MA, Pineda R et al. The anorexigenic neuropeptide, nesfatin-1, is indispensable for normal puberty onset in the female rat. *J Neurosci* 2010;30:7783-92.
- García-Galiano D, Pineda R, Ilhan T, Castellano JM, Ruiz-Pino F, Sánchez-Garrido MA et al. Cellular distribution, regulated expression, and functional role of the anorexigenic peptide, NUCB2/nesfatin-1, in the testis. *Endocrinology* 2012;153:1959-71.
- Ghanbari Niaki A, Mohammadi Joojadedeh F, Zare Kookandeh N, Najafi S, Chaichi MJ, Rodbari F et al. Liver and plasma nesfatin-1 responses to 6 weeks of treadmill running with or without zizyphus jujuba liquid extract in female rat. *Int J Endocrinol Metab* 2013;11:95-101.
- Goebel-Stengel M, Wang L. Central and peripheral expression and distribution of NUCB2/nesfatin-1. *Curr Pharm Des* 2013;19:6935-40.
- Gonzalez R, Perry RL, Gao X, Gaidhu MP, Tsushima RG, Ceddia RB et al. Nutrient responsive nesfatin-1 regulates energy balance and induces glucose-stimulated insulin secretion in rats. *Endocrinology* 2011;152:3628-37.
- Gonzalez R, Shepperd E, Thiruppugazh V, Lohan S, Grey C, Chang JP et al. Nesfatin-1 Regulates the Hypothalamo-Pituitary-Ovarian Axis of Fish. *Biol Reprod* 2012;87:84.
- Inhoff T, Stengel A, Peter L, Goebel M, Taché Y, Bannert N et al. Novel insight in distribution of nesfatin-1 and phospho-mTOR in the arcuate nucleus of the hypothalamus of rats. *Peptides* 2010;31:257-62.
- Ishida E, Hashimoto K, Shimizu H, Okada S, Satoh T, Kato I et al. Nesfatin-1 induces the phosphorylation levels of cAMP response element-binding protein for intracellular signaling in a neural cell line. *PLoS One* 2012;7:e50918.

- Jego S, Salvert D, Renouard L, Mori M, Goutagny R, Luppi PH et al. Tuberal hypothalamic neurons secreting the satiety molecule Nesfatin-1 are critically involved in paradoxical (REM) sleep homeostasis. *PLoS One* 2012;7:e52525.
- Jia FY, Li XL, Li TN, Wu J, Xie BY, Lin L. Role of nesfatin-1 in a rat model of visceral hypersensitivity. *World J Gastroenterol* 2013;19:3487-93.
- Kerbel B, Unniappan S. Nesfatin-1 suppresses energy intake, co-localises ghrelin in the brain and gut, and alters ghrelin, cholecystokinin and orexin mRNA expression in goldfish. *J Neuroendocrinol* 2012;24:366-77.
- Könczöl K, Pintér O, Ferenczi S, Varga J, Kovács K, Palkovits M et al. Nesfatin-1 exerts long-term effect on food intake and body temperature. *Int J Obes* 2012;36:1514-21.
- Li QC, Wang HY, Chen X, Guan HZ, Jiang ZY. Fasting plasma levels of nesfatin-1 in patients with type 1 and type 2 diabetes mellitus and the nutrient-related fluctuation of nesfatin-1 level in normal humans. *Regul Pept* 2010;159:72-7.
- Li Z, Gao L, Tang H, Yin Y, Xiang X, Li Y et al. Peripheral effects of nesfatin-1 on glucose homeostasis. *PLoS One* 2013;8:e71513.
- Maejima Y, Sedbazar U, Suyama S, Kohno D, Onaka T, Takano E et al. Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. *Cell Metab* 2009;10:355-65.
- Mimee A, Smith PM, Ferguson AV. Nesfatin-1 influences the excitability of neurons in the nucleus of the solitary tract and regulates cardiovascular function. *Am J Physiol* 2012;302:R1297-304.
- Oh-I S, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K et al. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. *Nature* 2006;443:709-12.
- Pałasz A, Krzystanek M, Worthington J, Czajkowska B, Kostro K, Wiaderkiewicz R et al. Nesfatin-1, a unique regulatory neuropeptide of the brain. *Neuropeptides* 2012;46:105-12.
- Perry B, Wang Y. Appetite regulation and weight control: the role of gut hormones. *Nutr Diabetes* 2012;2:e26.
- Ramanjaneya M, Chen J, Brown JE, Tripathi G, Hallschmid M, Patel S et al. Identification of nesfatin-1 in human and murine adipose tissue: a novel depot-specific adipokine with increased levels in obesity. *Endocrinology* 2010;151:3169-80.
- Shimizu H, Oh-I S, Hashimoto K, Nakata M, Yamamoto S, Yoshida N et al. Peripheral administration of nesfatin-1 reduces food intake in mice: the leptin-independent mechanism. *Endocrinology* 2009a;150:662-71.
- Shimizu H, Ohsaki A, Oh-I S, Okada S, Mori M. A new anorexigenic protein, nesfatin-1. *Peptides* 2009b;30:995-8.
- Shimizu H, Oh-I S, Okada S, Mori M. Nesfatin-1: an overview and future clinical application. *Endocr J* 2009c;56:537-43.
- Stengel A, Goebel M, Wang L, Rivier J, Kobelt P, Mönnikes H et al. Central nesfatin-1 reduces dark-phase food intake and gastric emptying in rats: differential role of corticotropin-releasing factor2 receptor. *Endocrinology* 2009;150:4911-9.
- Tan BK, Hallschmid M, Kern W, Lehnert H, Randeve HS. Decreased cerebrospinal fluid/plasma ratio of the novel satiety molecule, nesfatin-1/NUCB-2, in obese humans: evidence of nesfatin-1/NUCB-2 resistance and implications for obesity treatment. *J Clin Endocrinol Metab* 2011;96:E669-73.
- Vas S, Ádori C, Könczöl K, Kátai Z, Pap D, Papp RS et al. Nesfatin-1/NUCB2 as a potential new element of sleep regulation in rats. *PLoS One* 2013;8:e59809.
- Wang L, Stengel A, Goebel-Stengel M, Shaikh A, Yuan PQ, Taché Y. Intravenous injection of urocortin 1 induces a CRF2 mediated increase in circulating ghrelin and glucose levels through distinct mechanisms in rats. *Peptides* 2013;39:164-70.
- Yakabi K, Noguchi M, Ohno S, Ro S, Onouchi T, Ochiai M et al. Urocortin 1 reduces food intake and ghrelin secretion via CRF(2) receptors. *Am J Physiol Endocrinol Metab* 2011;301:E72-82.
- Yoshida N, Maejima Y, Sedbazar U, Ando A, Kurita H, Damdindorj B et al. Stressor-responsive central nesfatin-1 activates corticotropin-releasing hormone, noradrenaline and serotonin neurons and evokes hypothalamic-pituitary-adrenal axis. *Aging* 2010;2:775-84.
- Yosten GL, Samson WK. The anorexigenic and hypertensive effects of nesfatin-1 are reversed by pretreatment with an oxytocin receptor antagonist. *Am J Physiol* 2010;298:R1642-7.
- Zheng H, Lenard NR, Shin AC, Berthoud HR. Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals. *Int J Obes* 2009;33:S8-13.