Artykuł przeglądowy *Review*

Role of nesfatin-1 in the metabolism of skeletal tissues

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Summary

NUCB2/nesfatin-1, a member of the adipokine family, is a peptide hormone with pleiotropic action. It has been found in different tissues, including cartilage and bone cells. Nesfatin-1 is produced by chondrocytes, and its synthesis increases with the degree of cell differentiation and upon stimulation by pro-inflammatory cytokines, as shown in an in vitro study. An increase in serum levels of nesfatin-1 has been observed in humans with osteoarthritis, which indicates the influence of pro-inflammatory cytokines on nesfatin-1 release. On the other hand, nesfatin-1 stimulates the synthesis of pro-inflammatory cytokines by chondrocytes, which suggests its participation, together with other adipokines, in the pathogenesis and/or progression of inflammatory complications of cartilage degenerative diseases. Nesfatin-1 also promotes pre-osteoblastic cell differentiation and mineralization and inhibits macrophage differentiation towards osteoclasts. Moreover, exogenous nesfatin-1 given to ovariectomized rats reduces osteopenic changes. Therefore, it seems that nesfatin-1 may play a protective role in cartilage and bone diseases. However, further studies are required to determine whether nesfatin-1 can be used for monitoring and treatment of cartilage and bone diseases.

Keywords: nesfatin-1, NUCB2, bone, cartilage, skeletal tissue

Skeletal disorders are a serious problem in humans and animals. Since they are multifactorial diseases, their treatment is relatively difficult. Therefore, the exact knowledge of mechanisms regulating bone metabolism is important for choosing appropriate preventive procedures. Due to the severity of the problem, in recent years attention has been paid to the role of various factors in the physiology of bone and cartilage tissues. The current data indicate that some adipokines, e.g. adiponectin, visfatin, resistin, and the best-known leptin, exert a complicated and inconsistent effect on bone metabolism (4, 5, 8, 13, 28, 33, 40, 45, 57, 66). These findings are the basis for the claim that nesfatin-1 as an adipokine may play an important role in the metabolism of skeletal tissue as well.

Nesfatin-1, i.e. an 82-amino-acid peptide, was first described by Oh-I and co-workers in 2006 (38). It was found as an anorexigenic protein derived from nucleobindin-2 (NUCB2) and was named NUCB2-encoded satiety and fat-influencing protein/nesfatin-1. Therefore, nesfatin-1 is frequently called NUCB2.

Nucleobindin was identified in the 1990s as a protein capable of binding Ca^{2+} ions, located in the nucleus, the Golgi apparatus, and the endoplasmic reticulum (3, 35). Further investigations revealed two types of this protein: nucleobindin-1 (NUCB1) and nucleobindin-2 (NUCB2). NUCB2 has a 24-amino acid signal peptide at the N-terminus and a chain consisting of 396 amino acids characterized by a constant sequence in rodents and humans. As a result of post-translational changes in the NUCB2 molecule in the presence of prohormone convertase (PC) -1/3, three peptides are distinguished: nesfatin-1 (residues 1-82), nesfatin-2 (residues 85-163), and nesfatin-3 (residues 166-396) (47). It has been found in studies on these peptides that only nesfatin-1 shows biological activity, and its effect is based on the inhibition of food intake in rats after either peripheral or central administration (14, 17, 21, 38, 47, 49, 50).

Location of nesfatin-1 in the body

Initially, NUCB2/nesfatin-1 mRNA expression was found in neurons of the hypothalamus and brainstem, such as the paraventricular nucleus, supraoptic nucleus, arcuate nucleus, lateral hypothalamus, zona incerta, and solitary tract nucleus. Immunohistochemical stud-

ies showed the same location of nesfatin-1 (6, 38). In fact, NUCB2/nesfatin-1 is abundantly expressed in several regions of the hypothalamus that play key roles in controlling food intake. It is now known that immunopositive reaction to NUCB2/nesfatin-1 also occurs in other areas, including the periventricular nucleus, Edinger-Westphal nucleus, dorsal nucleus of the vagus nerve, insular cortex, cerebellum as well as sympathetic and parasympathetic neurons of the spinal cord within the thoracic, lumbar, and sacral parts (6, 14, 16, 20, 24, 31, 38, 62). Within neurons, nesfatin-1 occurs in conjunction with different neurotransmitters, including primarily melanin (MEL), cocaine- and amphetamine-regulated transcript (CART), proopiomelanocortin (POMC), α-melanocyte-stimulating hormone (α-MSH), growth hormone-releasing hormone (GHRH), thyrotropin-releasing hormone (TRH), corticotropin-releasing hormone (CRH), oxytocin (OT), vasopressin (AVP), neurotensin (NT), acetylocholine (Ach), and serotonin (SER) (6, 12, 14, 16, 31, 34, 39). The anatomical location of neurons expressing nesfatin-1 and its coexistence with neurotransmitters suggests that the physiological role of nesfatin-1 is not limited to the regulation of food intake, but also includes the regulation of neuroendocrine and autonomic control of internal organs and emotional reactions (14, 22, 34).

The expression of NUCB2/nesfatin-1 mRNA was also observed in peripheral tissues, including the stomach, duodenum, pancreas, heart, adipose tissue, and testis (19, 23, 44, 48, 65). Gastric NUCB2/nesfatin-1 mRNA expression levels were higher than expression levels in other peripheral organs and brain (48). Nesfatin-1 reduced the release of gastric acid in a dose-dependent manner and influenced gastrointestinal motility (2, 24, 55, 58). Its presence in pancreatic cells was detected particularly in the beta cells of Langerhans islets (15, 27). Nesfatin-1 stimulates the release of insulin in response to glucose and increases mRNA expression of preproinsulin in isolated islets of Langerhans in rats and mice via an intracellular signal associated with the influx of Ca^{2+} through long-term L-type calcium channels (26, 36). Intravenous nesfatin-1 injections lower plasma glucose in obese mice with phenotype db/db as well as in non-diabetic and non-obese mice (51). Moreover, elevated glucose in blood increases the release of nesfatin-1 from endocrine cells of the pancreas (11), suggesting that nesfatin-1 may affect the control of blood glucose by insulin. However, as shown by some researchers, nesfatin-1 serves only a local function in the pancreas (7).

Nesfatin-1 also exerts other effects on the organism. This peptide influences water intake (64), energy expenditure (56), sleep (54), blood pressure (61, 63), and reproduction (18, 19). Serum nesfatin-1 levels correlated positively with increasing BMI, demonstrating that systemic nesfatin-1 levels may be influenced by the body fat (1, 44). Human and murine adipocytes are capable of producing nesfatin-1, which confirms that this anorexigenic molecule is an adipokine. Hence, a potential source of peripheral nesfatin-1 is white adipose tissue, especially the subcutaneous tissue (44).

Location and function of nesfatin-1 in bone and cartilage

The first studies concerning the interaction of nucleobindin and bone indicated that this protein was a product of bone cells, osteoblasts and osteocytes, and that it was secreted into the osteoid (42). Its intracellular and extracellular location was found. However, immunohistochemistry analysis detected the most intense nucleobindin labeling extracellularly in newly formed bone in growing rats, whereas in compact bone, labeling for nucleobindin was located in osteocytes. The expression of nucleobindin was up-regulated during differentiation and matrix maturation, and subsequently down-regulated during mineralization (42). These observations suggest that nucleobindin might play a role as a modulator of matrix maturation and might be important for transcellular Ca^{2+} transport during mineralization. The studies mentioned above were the basis for further research. However, at present, research on the role of nesfatin-1 in bone and cartilage physiology is very limited. Preliminary papers in this field date from 2013 and later.

Jiang et al. (30) demonstrated nesfatin-1 gene expression in articular cartilage, osteophytes, meniscus, synovium, and intrapatellar fat pads in samples of osteoarthritis cartilage and non-osteoarthritis cartilage. The higher mRNA nesfatin-1 expression in osteoarthritis cartilage indicates that nesfatin-1 may be involved in the pathogenesis of osteoarthritis. Immunohistochemical analysis indicated that all osteoarthritis cartilage, osteophyte, and synovium contained nesfatin-1. In the synovium and osteophytes, nesfatin-1 was present notably in the superficial layers. Nesfatin-1 staining was stronger in lesional areas of cartilage than in non-lesional areas. Significantly elevated levels of nesfatin-1 in patients with osteoarthritis, compared with serum from healthy controls, were also confirmed (30, 67). Furthermore, correlations were observed between serum nesfatin-1 and high sensitivity C-reactive protein (hsCRP) levels and between synovial nesfatin-1 and IL-18 (interleukin-18) levels in osteoarthritis patients. IL-18 and hsCRP are associated with cartilage inflammation (29, 41). Thus, the relationship of IL-18, hsCRP, and nesfatin-1 may indicate that nesfatin-1 could be a novel marker of osteoarthritis. Moreover, as reported by Zhang et al. (67), serum and synovial fluid nesfatin-1 concentrations were associated with osteoarthritis development. On the other hand, the fact that the nesfatin-1 level in serum, observed by Jiang et al. (30), was significantly higher than it was in synovial fluid indicates that circulating levels of nesfa-

tin-1 do not accurately represent the situation in joint tissues.

As shown in an *in vitro* study, the expression of NUCB2 mRNA and NUCB2/nesfatin-1 protein was detected in human primary chondrocytes and murine ATD-5 chondrocytes (46). The cytoplasmic location of nesfatin-1 in these cells was also demonstrated. Furthermore, mRNA and protein expression was increased during maturation of chondrocytes, which suggests that nesfatin-1 may play a role in chondrocyte differentiation and cartilage maturation. It might also be a marker of late-phase chondrogenic differentiation and might affect endochondral ossification. The increase observed in its expression during the maturation of chondrocytes confirms previous assumptions by Petersson et al. (42). Nesfatin-1 can also induce chemotactic and pro-inflammatory mediators, such as interleukin-8 (IL-8), interleukin-6 (IL-6), cyclooxygenase-2 (COX-2), and macrophage inflammatory protein-1 alfa (MIP-1 alfa), in human primary chondrocytes (46). It also induces IL-6, MIP-1 alfa, and COX-2 in murine differentiated chondrocytes in combination with IL-1 (46). On the other hand, IL-1 and TNF-alfa (tumor necrosis factor-1) increase NUCB2 mRNA expression as well as NUCB2/nesfatin-1 synthesis in human primary chondrocytes and murine differentiated chondrocytes. As indicated by the research, pro-inflammatory cytokines in chondrocytes are also induced by other adipokines. Conde et al. (9, 10) and Gomez et al. (25) have shown that leptin and adiponectin participate in inflammation and immune response in whole joint tissues and in rheumatic diseases, such as osteoarthritis and/or rheumatoid arthritis. Thus, these findings suggest that nesfatin-1 may participate, together with other adipokines, in the pathogenesis and/or progression of inflammatory complications of cartilage degenerative diseases. Moreover, it seems that nesfatin-1 may play a protective role or respond to osteoarthritis.

Research concerning the role of nesfatin-1 in bone metabolism and properties has been very limited. Li et al. (32) found the expression of NUCB2/nesfatin-1 mRNA in osteoblast and osteoclast cell lines in an *in vitro* study. It was also demonstrated that NUCB2/nesfatin-1 significantly increased the activity of alkaline phosphatase (ALP), a representative marker of osteoblastic differentiation, in pre-osteoblastic cells in a dose-dependent manner. However, this effect was found to be dependent on recombinant human bone morphogenetic protein-2 (rhBMP2), since NUCB2/nesfatin-1 itself had no direct effect on the ALP activity. In contrast, NUCB2/nesfatin-1 improved mineralization with no addition of rhBMP2. Moreover, NUCB2/nesfatin-1 reduced osteoclastogenesis by inhibiting macrophage differentiation towards osteoclasts (32). The influence of nesfatin-1 on bone cells is similar to the effect of other adipokines, e.g. visfatin stimulates osteoblast proliferation and inhibits osteoclastogenesis, whereas resistin stimulates both osteoblast proliferation and osteoclast differentiation (36, 52, 59). Adiponectin alters bone metabolism, promoting bone formation and inhibiting bone resorption (33, 40, 60). It also influences chondrocyte proliferation, matrix synthesis, and mineralization (8). The leptin effect on bone metabolism is not fully clear. As demonstrated in the same studies, leptin has a protective effect on bone formation and bone mineral content (4, 5); on the other hand, it inhibits bone formation through the nervous system (13).

In an *in vivo* study, intravenous administration of NUCB2/nesfatin-1 was found to increase bone mineral density of femora and lumbar vertebrae in ovariectomized rats, which are a classic animal model for postmenopausal osteoporosis (32). In another study conducted in female rats undergoing ovariectomy, exogenous nesfatin-1 protected the growth cartilage of long bones. The thickness of the growth plate in ovariectomized rats after nesfatin-1 treatment was similar to that in control animals (53).

As mentioned earlier, nesfatin-1 can induce pro-inflammatory cytokines, and the nesfatin-1 serum level is elevated in osteoarthritis incidences. Elevated serum nesfatin-1 levels were also connected with osteopenic changes observed after gastric resection (43). Pro-inflammatory cytokines, in addition to arthritis and cartilage disorders, are also involved in bone remodeling and pathogenesis of osteoporosis. They are key regulators of osteoclastogenic activity and stimulate bone resorption, leading to bone loss and negative changes in its structure and properties. Given the above, it can be supposed that the increase in nesfatin-1 in blood may be a protective mechanism against changes in osteopenic bone. A confirmation of this thesis may be the protective effect of nesfatin-1 on bone tissue in ovariectomized rats (32, 53).

In summary, it seems that nesfatin-1 may play a protective role in cartilage and bone diseases, but the mechanism of its action is ambiguous. Therefore, further studies are required to investigate the exact role of nesfatin-1 and to determine whether this adipokine can be used for monitoring and treatment of cartilage and bone diseases.

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