Current Understanding of the Role of Nesfatin-1

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Nesfatin-1 was discovered in 2006 and implicated in the regulation of food intake. Subsequently, its widespread central and peripheral distribution gave rise to additional effects. Indeed, a multitude of actions were described, including modulation of gastrointestinal functions, glucose and lipid metabolism, thermogenesis, mediation of anxiety and depression, as well as cardiovascular and reproductive functions. Recent years have witnessed a great increase in our knowledge of these effects and their underlying mechanisms, which will be discussed in the present review. Lastly, gaps in knowledge will be highlighted to foster further studies.

Nesfatin-1 is an 82–amino acid polypeptide derived from the precursor protein nucleobindin 2 (NUCB2), whose processing also yields nesfatin-2 and -3, two peptides with so far unknown functions [1]. The 29-amino acid mid-fragment of nesfatin-1, nesfatin-1$_{30-59}$, has been identified as the active core of nesfatin-1, also exerting an anorexigenic effect [2]. Although full-length nesfatin-1 contains cleavage sites at the respective amino acids, cleavage has not been shown *in vivo*.

NUCB2/nesfatin-1 (most antibodies do not distinguish between NUCB2 and processed nesfatin-1, and therefore we refer to the analyte as NUCB2/nesfatin-1) was first detected in food intake regulatory brain nuclei such as the paraventricular nucleus, arcuate nucleus, and nucleus of the solitary tract [1], and subsequent studies extended this distribution to numerous other brain nuclei in the rat [3] and mouse [4]. It is to note that NUCB2/nesfatin-1 has been detected primarily in the soma and primary dendrites of neurons, whereas less immunoreactivity was observed in nerve fibers [5], possibly pointing to an autocrine or paracrine rather than endocrine mode of action. Nonetheless, nesfatin-1 was shown to cross the blood-brain barrier in both directions [6, 7], supporting a humoral route of signaling. Further corroborating this assumption, NUCB2/nesfatin-1 has also been detected in the periphery, namely in the gastric mucosa [8], adipose tissue [9], pancreatic beta cells [10], testes [11], ovaries [12], uterus, epididymis [13] and cardiomyocytes [14]. The stomach was identified as main source of peripheral NUCB2/nesfatin-1, with NUCB2 mRNA levels higher

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than in other peripheral organs or the brain [8]. Additional analyses indicated the expression of NUCB2/nesfatin-1 in gastric endocrine X/A-like cells coexpressed with the food intake-stimulating hormone ghrelin [8] in rats, a finding later confirmed in humans [15].

Despite the increasing knowledge of the expression and regulation of the ligand, the receptor mediating nesfatin-1's actions has not been identified. A recent study using auto-radiography showed nesfatin-1 binding in the brain, including the cortex, paraventricular nucleus of the hypothalamus, area postrema, dorsal motor nucleus of the vagus nerve, and cerebellum as well as in peripheral endocrine organs, namely the pituitary, stomach, small intestine, pancreas, adrenal gland, testes, and visceral adipose tissue and also in the heart, skeletal muscle, lungs, liver and kidneys [16]. This widespread binding of ^{125}I-nesfatin-1 as a surrogate for the putative expression of the receptor further supports the assumption of a pleiotropic action of the peptide. On a molecular level evidence points toward the mediation of nesfatin-1's effects *via* a G_i protein-coupled receptor with nesfatin-1 inducing a Ca^{2+} inflow through L-type, *N*-type, or P/Q-type Ca^{2+} channels [17, 18] and promoting the phosphory-lation of cAMP responding element–binding protein (CREB) in neuroblastoma cells *in vitro,* additionally stimulating mitogen-activated protein kinase signaling [19]. In contrast, in cardiomyocytes G_q is the supposed receptor type because nesfatin-1 suppressed L-type Ca^{2+} channel functioning by means of protein kinase C [20].

In the present review we describe the pleiotropic effects of nesfatin-1 and highlight un-derlying mechanisms with a focus on recent developments. We also discuss gaps in knowledge to demonstrate the need for further studies. A PubMed search has been performed and all studies with the terms "nesfatin-1," "NUCB2," and "nucleobindin 2" screened.

1. Implications of Nesfatin-1 in the Regulation of Food Intake

In the landmark study of Oh-I *et al.* [1], nesfatin-1 was described as anorexigenic peptide exerting a robust reduction of dark phase food intake after third ventricular injection, with a reduction of body weight gain after repeated injection. This effect is likely to be of physio-logical importance because blockade of endogenous nesfatin-1 with an antisense oligonu-cleotide [1] or knockdown of NUCB2 specifically in the paraventricular nucleus of the hypothalamus [21] resulted in a stimulation of food intake and a subsequent increase in body weight in rats [1, 21]. The anorexigenic effect after brain ventricular injection was confirmed in subsequent studies by independent groups in rats [22–24], mice [25, 26], pigs, [27], and goldfish [28], pointing toward a robust effect. Interestingly, a recent study using mice with NUCB2 knockout in several brain areas and peripheral tissues failed to demonstrate an effect on food intake and body weight compared with control mice, leading to the conclusion by the authors that nesfatin-1 does not affect food intake [29]. However, the authors did not check the expression of NUCB2/nesfatin-1 in the stomach, a major source of the peptide as described earlier. This expression might well explain the circulating levels of NUCB2/nesfatin-1 still observed in these mice. Lastly, the possibility of a compensatory action by other food intake regulatory peptides under conditions of long-term loss of a hormone also must be kept in mind.

In contrast to the brain effects, conflicting data exist on the peripheral effects of nesfatin-1, with some studies showing no effect [24], whereas others report a decrease in food intake after intraperitoneal (IP) injection of high doses in mice [2]. Similarly, chronic subcutaneous in-fusion of nesfatin-1 at high doses *via* osmotic minipumps was able to inhibit food intake in rats [30]. Overall, the anorexigenic effect is more readily observed after central injection of low doses of the peptide, giving rise to a predominantly central mode of anorexigenic action.

In addition to the food intake-reducing effect, intracerebroventricularly (ICV) injected nesfatin-1 was also shown to reduce water intake, an effect likely to present a physiological action of the peptide because pretreatment with an antinesfatin-1 antisense oligonucleotide led to an increased drinking response to angiotensin II [31].

Subsequent studies investigated the microstructure underlying the reduction of food intake by using an automated system that allows the continuous monitoring of food intake in

undisturbed rodents. In mice, ICV injected nesfatin-1 reduced meal size, indicating increased satiation and meal frequency associated with a prolongation of intermeal intervals, reflecting higher satiation [25]. Interestingly, ICV injected nesfatin-1$_{30-59}$, described as the active core of nesfatin-1 [2], increased satiety as well whereas satiation was not altered in mice [32], giving rise to differential receptor binding or activation. Lastly, in rats nesfatin-1$_{30-59}$ induced satiation but not satiety [33], pointing toward species differences, a finding confirmed after microinjection of nesfatin-1 into the lateral parabrachial nucleus in rats [34]. Although the effect of nesfatin-1$_{30-59}$ was retained during diet-induced obesity (DIO) in rats [33], giving rise to a leptin-independent signaling as suggested before [1, 2], the underlying micro-structure differed, with increased satiety [33] suggesting different downstream signaling under conditions of obesity.

When nesfatin-1 was injected ICV in rats, its anorexigenic effect was abolished by pre-treatment with the selective corticotropin-releasing factor (CRF) 2 antagonist astressin$_2$-B [24] indicating downstream mediation *via* corticotropin-releasing factor receptor 2 (CRF$_2$) signaling. This finding was also confirmed in chicks with astressin-B, a CRF$_{1/2}$ antagonist [35]. Because the melanocortin receptor 3/4 antagonist SHU9119, with anorexigenic melanocortin 4 signaling being well established upstream of CRF [36, 37], attenuated [34] or blocked [1] nesfatin-1's anorexigenic effect, nesfatin-1 might act *via* melanocortin → CRF signaling to inhibit food intake.

Moreover, nesfatin-1 has been shown to inhibit neuropeptide Y (NPY)-containing cells *in vitro* [38]. Because NUCB2/nesfatin-1 partly colocalizes with NPY in the arcuate nucleus [39], this effect might involve an autocrine mode of signaling. Also, mammalian target of rapamycin (*m*-TOR) is greatly colocalized with NUCB2/nesfatin-1 in the arcuate nucleus [39]. Based on the finding that *m*-TOR has been shown to reduce NPY mRNA expression [40], nesfatin-1 might also signal *via* this pathway in an endocrine manner to reduce food intake. Peripherally, a decrease of *m*-TOR signaling has been shown to reduce the expression of NUCB2/nesfatin-1 *in vitro* and *in vivo* [41], whereas an increase of gastric phosphorylated mammalian target of rapamycin (pm-TOR)/*m*-TOR expression was associated with an increased gastric secretion of NUCB2/nesfatin-1, resulting in elevated levels of circulating NUCB2/nesfatin-1 [42] probably involved in the observed suppression of food intake. Interestingly, in the brain a decrease of pm-TOR levels in the dorsal motor nucleus of the vagus nerve induced by fourth ventricular injection of nesfatin-1 was associated with a reduction of food intake [43], pointing toward a differential central or peripheral regulation of *m*-TOR.

Also, fibroblast growth factor 21 (FGF21) is likely to be involved in the mediation of nesfatin-1's anorexigenic effect because FGF21 increased the expression of NUCB2 mRNA in the paraventricular nucleus, increased [Ca^{2+}]$_i$ in NUCB2/nesfatin-1-containing neurons, and the suppression of food intake induced by ICV injection of FGF21 was absent in NUCB2 knockout mice [44].

Nesfatin-1 has been recently implicated in the endogenous inhibition of food intake induced by IP application of cisplatin because cisplatin-activated NUCB2/nesfatin-1 neurons in the hypothalamus and brainstem and the cisplatin-induced reduction of food intake were attenuated by ICV injection of nesfatin-1/NUCB2-antisense [45], giving rise to a patho-physiological role of nesfatin-1 in chemotherapy-induced hypophagia. Similarly, nesfatin-1 has also been implicated in the oxytocin-induced suppression of food intake because IP injected oxytocin increased the number of activated NUCB2/nesfatin-1 neurons in the paraventricular nucleus, arcuate nucleus, and nucleus of the solitary tract, and the oxytocin-induced anorexigenic effect was attenuated by ICV injection of antisense nesfatin-1 [46]. Conversely, ICV injection of nesfatin-1 activated oxytocin-positive neurons in the para-ventricular nucleus of the hypothalamus, as assessed with c-Fos and stimulated the release of oxytocin *in vitro* [47]. Lastly, an oxytocin receptor antagonist blocked the anorexigenic effect of nesfatin-1 [22, 47] indicating a strong interaction between nesfatin-1 and oxytocin in the inhibition of food intake.

Repeated injections of nesfatin-1 into the third brain ventricle [1] or the lateral para-brachial nucleus [34] decreased body weight gain, probably *via* a stimulation of uncoupling protein 1 (UCP1) expression in brown adipose tissue [34].

The regulation of NUCB2/nesfatin-1 supports a role for this peptide as a physiological modulator of food intake. Whereas fasting led to a decrease in hypothalamic NUCB2 mRNA levels [1, 48], refeeding activated NUCB2/nesfatin-1-positive neurons in the supraoptic nucleus, associated with an increase in NUCB2 mRNA levels [48]. In line with these findings, plasma NUCB2/nesfatin-1 levels decreased during 24-hour fasting and were restored after refeeding in rats [8]. *In vitro*, glucose elevated NUCB2 mRNA expression and NUCB2/nesfatin-1 secretion from cultured stomach ghrelinoma cells [49], probably contributing to the observed increase in plasma levels. Interestingly, in goldfish intestinal cells NUCB2 mRNA was decreased by glucose *in vitro* [50], pointing toward species differences. Also, sex differences seem to play a role because male mice receiving a high-fat diet displayed a decrease in serum NUCB2/nesfatin-1 levels [49], whereas this diet did not alter gastric NUCB2 mRNA expression in female mice [51]. Lastly, in zebrafish receiving a high-fat diet, supplementation with *Lactobacillus rhamnosus* reduced gut NUCB2/nesfatin-1 levels [52], pointing toward a microbiota-associated regulation of nesfatin-1 to be further investigated.

In obese children, no difference in NUCB2/nesfatin-1 plasma levels was detected between the fasting and postprandial state [53]. Whether this also holds true under normal weight conditions remains to be investigated. Moreover, an investigation of dynamic meal-related changes in circulating NUCB2/nesfatin-1 in humans is still lacking. Chronic changes in body weight also affect the levels of circulating NUCB2/nesfatin-1, with decreased levels in underweight and anorexic subjects [54, 55]. Because rats with activity-based anorexia, an animal model for anorexia nervosa, showed an activation of nesfatin-1 immunoreactive neurons in several brain nuclei involved in the mediation of food intake, gastrointestinal functions, and the response to stress [56], nesfatin-1 might be implicated in the development or maintenance of this condition, a hypothesis to be further investigated.

Conversely, circulating NUCB2/nesfatin-1 levels were shown to be elevated in obese adults [9, 57], probably because of increased expression of NUCB2/nesfatin-1 in the stomach [15]. These circulating levels were observed to be decreased after bariatric surgery–associated weight loss [58]. However, other studies described a negative association of circulating NUCB2/nesfatin-1 and body mass index (BMI) [55, 59, 60] and an increase after bariatric surgery in humans [61] and also mice [62], giving rise to additional, confounding factors such as sex or comorbidities like diabetes. Also, chronic malnutrition in children was reported to upregulate serum NUCB2/nesfatin-1 concentrations [63]. Whether this effect reflects metabolism- or stress-associated changes remains to be investigated.

2. Implications of Nesfatin-1 in Gastrointestinal Functions

As observed earlier for several peptides regulating food intake, nesfatin-1 has been shown to alter gastrointestinal functions. Early on, an inhibitory action of nesfatin-1 on antral and duodenal motility [26] and a reduction of gastric emptying was shown after ICV injection [24], a finding later also observed after microinjection of the peptide into the central nucleus of the amygdala [64], basomedial amygdala [65], arcuate nucleus [66], paraventricular nucleus of the hypothalamus [67], lateral hypothalamus [68], and ventromedial hypothalamus in rodents [69], giving rise to the sites of action. Because gastric distension induced c-Fos expression in NUCB2/nesfatin-1-positive neurons not only in the nucleus of the solitary tract [70] but also in the ventromedial hypothalamus [71], and the injection of an antinesfatin-1 antibody prevented a nucleus accumbens-induced ventromedial hypothalamic neuronal firing [69], along with the finding that rats with lesioned ventromedial hypothalamic nuclei showed increased gastric emptying [72], ventromedial nucleus nesfatin-1 signaling might well play a role in the endogenous regulation of gastric motility.

Because intravenous (IV) application of nesfatin-1 was able to reduce gastric contractions in dogs [73], the effect might also be mediated in the periphery, as recently hypothesized, by the distribution of the receptor as assessed by autoradiography [16]. A recent study reported that chronic gastric electrical stimulation used as a novel attempt to treat intractable vomiting and nausea reduced circulating NUCB2/nesfatin-1 levels [74], possibly indicating

nesfatin-1 as pathophysiological contributor to this disease. This hypothesis should be followed up in future studies.

Besides an effect on motility, nesfatin-1 affects secretory functions: ICV injected nesfatin-1 reduced 2-deoxy-D-glucose-stimulated gastric acid production [75] associated with a lower expression of H^+/K^+-ATPase in obese rats [76]. Moreover, nesfatin-1 seems to be implicated in inflammatory processes, because IP [77] or IV [78] injected nesfatin-1 improved healing in rat models of gastric ulcer, associated with a decrease in TNF-α and IL-1β and mediated by downstream cyclooxygenase 2 signaling [79, 80].

3. Implications of Nesfatin-1 in Blood Glucose Homeostasis

Early on, an implication of nesfatin-1 in glucose homeostasis was suggested. Besides its expression in the stomach, NUCB2/nesfatin-1 has also been detected in endocrine islets of the pancreas [8, 81, 82] colocalized with insulin in rodents [10, 83], dogs [84], pigs [84], and humans [85].

In rats, glucose challenge led to a release of NUCB2/nesfatin-1 from isolated pancreatic islets *in vitro* [86], probably by activating L-type Ca^{2+} channels [87] and inhibiting $K_v2.1$ channels [88]; however, the release of insulin was more pronounced [83]. Nesfatin-1 further increased the glucose-stimulated insulin release *in vitro* [86], giving rise to a direct effect supported by the assumed nesfatin-1 receptor in the pancreas as suggested by autoradiography [16], an effect leading to a reduction in glucose levels in mice [85, 89]. The glucose-lowering effect after oral glucose intake [90] probably also involves a nesfatin-1-induced release of glucagon-like peptide 1, because preproglucagon mRNA was upregulated by nesfatin-1 in the enteroendocrine STC-1 cell line *in vitro* [91]. Moreover, subcutaneously infused nesfatin-1 increased insulin-stimulated phosphorylation of protein kinase B (AKT) in skeletal muscle, adipose tissue, and liver and glucose transporter type 4 membrane translocation in skeletal muscle and adipose tissue of mice [92]. IV infusion of nesfatin-1 in streptozotocin-induced type 2 diabetic mice decreased blood glucose and insulin resistance by increasing the expression of phosphorylated 5' AMP-activated protein kinase (*p*-AMPK) and acetyl-CoA carboxylase (*p*-ACC) of skeletal muscles [93]. Conversely, knockdown of hypothalamic NUCB2/nesfatin-1 increased hepatic glucose flux and reduced glucose uptake of peripheral tissues under conditions of normal weight and in obese rats, effects associated with decreased hepatic insulin receptors, insulin receptor substrate 1, and AKT kinase phosphorylation and phosphorylation of *m*-TOR, signal transducer and activator of transcription 3 (STAT3), and the suppressor of cytokine signaling 3 [94]. Simultaneously, hepatic glucose-6-phosphatase and phosphoenolpyruvate carboxykinase levels were increased [94]. Lastly, ICV injection of nesfatin-1 led to increased muscle glucose absorption, insulin receptor signaling through the AKT/AMP-activated protein kinase (AMPK)/transducer of regulated CREB protein 2 pathway, decreased gluconeogenesis and hepatic mRNA and protein expression and activity of phosphoenolpyruvate carboxykinase [95].

In immunohistochemical studies using c-Fos as marker for neuronal activity, hypoglycemia after peripheral insulin administration induced an activation of nesfatin-1-expressing neurons in the arcuate nucleus, paraventricular nucleus, lateral hypothalamic area, dorsal motor nucleus of the vagus, and nucleus of the solitary tract [96]. Retrograde tracing with fluorogold showed that those activated neurons in the dorsal motor nucleus of the vagus project to the pancreas and stomach, suggesting that nesfatin-1 is implicated in central glucose sensing and initiates the hormonal and physiological response to hypoglycemia [96]. Accordingly, nesfatin-1 inhibited almost 90% of the gastric distension inhibitory and activated >75% of gastric distension excitatory neurons in the dorsal vagal complex [97]. Similarly, nesfatin-1 injected into the lateral parabrachial nucleus activated the majority of glucose-inhibited glucosensing neurons [34]. Moreover, in the paraventricular nucleus glucose and insulin increased calcium concentrations in nesfatin-1 immunoreactive neurons, leading to activation of those initiating satiety [98]. In contrast, in the nucleus of solitary tract nesfatin-1 did not trigger the response of glucosensing neurons when the glycemic state changed [99].

Nesfatin-1's expression was altered under conditions of type 2 diabetes mellitus with reduced pancreatic NUCB2/nesfatin-1 peptide expression in rodents with genetically determined diabetes [83], whereas in DIO mice the NUCB2/nesfatin-1 peptide concentration was increased [86]. In humans, NUCB2 mRNA expression was reduced in islets of subjects with type 2 diabetes [85]. However, circulating NUCB2/nesfatin-1 levels were not altered under conditions of type 2 diabetes in rats [83], whereas in streptozotocin-induced type 2 diabetic mice [93] and in humans levels were higher in patients with type 2 diabetes or impaired glucose tolerance than in healthy controls [100], giving rise to species differences. However, in patients with metabolic syndrome, including insulin resistance, NUCB2/nesfatin-1 concentrations were lower than in controls [101]. A recent meta-analysis failed to show differences in circulating NUCB2/nesfatin-1 concentrations between controls and patients with type 2 diabetes [102]. Subgroup analyses showed significantly higher levels in patients newly diagnosed with type 2 diabetes without any treatment and lower levels in patients receiving antidiabetic medication [102], indicating an influence of medication on NUCB2/nesfatin-1 signaling. In line with this assumption, in a study of 100 patients with type 2 diabetes mellitus the oral glucose-lowering agent saxagliptin increased circulating NUCB2/nesfatin-1 along with C-peptide and improved insulin resistance as well as metabolic profile, BMI and blood pressure [103]. The observed negative correlation between nesfatin-1 and homeostatic model assessment for insulin resistance and hemoglobin A1c [103] emphasizes the beneficial effect of nesfatin-1 on glucose homeostasis.

The c.1012C>G polymorphism of the NUCB2 gene has been associated with a reduced risk of type 2 diabetes in the Chinese Han population [104]. In patients with type 2 diabetes mellitus the GG phenotype was related to lower BMI and fasting plasma glucose levels [104]. In accordance, in patients with metabolic syndrome the CG and GG genotypes were found less frequently, giving rise to a reduced risk of metabolic syndrome associated with the GG genotype and G allele [105].

In patients with gestational diabetes mellitus serum NUCB2/nesfatin-1 concentrations, NUCB2/nesfatin-1 levels in cord blood and protein expression of NUCB2/nesfatin-1 in subcutaneous fat were higher than in healthy pregnant subjects [106]. Inconsistently, a previous [107] and a subsequent [108] study showed the opposite regulation, with decreased serum NUCB2/nesfatin-1 levels and a positive association between NUCB2/nesfatin-1 and gestational week [108]. These discrepant results might be due to, although subtle, differences in age (29.6 $\pm$ 5.3 $vs.$ 32.1 $\pm$ 6.2 years), BMI (31.0 $\pm$ 5.5 $vs.$ 33.8 $\pm$ 6.5 kg/m^2), and advancement of pregnancy (26.2 $\pm$ 1.8 $vs.$ 23.0 $\pm$ 7.8 weeks), to be considered in future studies.

Laparoscopic Roux-en-Y gastric bypass or sleeve gastrectomy was shown to reduce BMI, waist circumference, hip circumference, waist-to-hip ratio, fasting blood glucose, and hemoglobin A1c levels, homeostatic model assessment for insulin resistance, and plasma insulin C-peptide after 1 year in patients with type 2 diabetes [109]. Glucagon stimulation reduced plasma levels of NUCB2/nesfatin-1, with higher levels observed in nonremitters compared with remitters [109]. Whether nesfatin-1 plays a pathogenetic role under these conditions or is compensatorily increased to improve glucose homeostasis will have to be further investigated in longitudinal studies.

4. Implications of Nesfatin-1 in Energy Metabolism

It was shown early on that nesfatin-1 is able to elevate core body temperature in rats after ICV injection [23], also modulating energy expenditure, dry heat, brown adipose tissue growth, and tail temperature, probably mediated via downstream melanocortin 3/4 signaling [110]. Moreover, cold ambient temperature activated nesfatin-1 immunoreactive neurons in the paraventricular nucleus [23]. Because knockout of the transcription factor Yin Yang 1 increased energy expenditure and oxygen consumption in beige and white fat depots along with an increased expression of NUCB2 mRNA in brown adipose tissue [111], NUCB2/nesfatin-1 might be physiologically involved in the regulation of energy expenditure. Lastly, nesfatin-1 was shown to stimulate brown adipocyte differentiation via m-TOR signaling and

upregulation of UCP1 mRNA expression [112]; therefore, nesfatin-1 might well be involved in long-term changes of energy expenditure. Because nesfatin-1 reduces food intake and increases energy expenditure, it induces a negative energy balance, which might be relevant in states of overnutrition but also might reflect conditions of stress, ultimately leading to wasting or cachexia, a hypothesis to be further investigated.

With regard to lipid metabolism, chronic subcutaneous infusion of nesfatin-1 reduced cholesterol and triglyceride levels in mouse plasma, an effect independent of food intake and body weight [113]. In diabetic mice, IV injected nesfatin-1 was able to normalize free fatty acids and body weight after 6 days, accompanied by an elevation of p-AMPK and p-ACC expression levels in skeletal muscle, indicating improved free fatty acid utilization [93]. Moreover, lipid accumulation was reduced by nesfatin-1 in cultured hepatocytes *in vitro,* probably because of a decrease of lipogenesis-relevant genes such as peroxisome proliferator-activated receptor-γ and sterol-regulatory element-binding protein 1 and enzymes including fatty acid synthase and glycerol-3-phosphatase acyltransferase, whereas β-oxidation-related genes were increased [113]. Because circulating levels of NUCB2/nesfatin-1 were reduced in patients with nonalcoholic fatty liver disease [114], the peptide might play a pathogenetic role under these conditions. This role should be further investigated along with the therapeutic potential in this disease.

5. Implications of Nesfatin-1 in the Development of Anxiety and Depression

Nesfatin-1 was shown to exert an anxiogenic effect after ICV injection in rats [115], a finding subsequently confirmed after IP injection [116]. A rat model inducing anxiety by using maternal separation and acute gastric irritation in early life and sequential stress in adulthood displayed increased NUCB2/nesfatin-1 peptide levels in the hippocampus, gastric fundus, and plasma, possibly contributing to the increased anxiety under these conditions [117]. Interestingly, in humans a correlation between circulating NUCB2/nesfatin-1 levels and self-reported anxiety was observed, with a positive association in women [118, 119], whereas in men this association was negative [120], pointing toward a sex-specific regulation of the peptide. However, the reduction of anxiety under inpatient treatment conditions did not significantly alter circulating NUCB2/nesfatin-1 levels [121]. Whether a longer (>4 weeks) observation time is necessary or whether patients with more severe anxiety disorders should be included to detect changes in NUCB2/nesfatin-1 warrants further investigation. Because anxiety and food intake are intimately linked, it might also be speculated that nesfatin-1 primarily affects anxiety and subsequently food intake. This speculation should be followed up, for example, by assessment of food intake after coapplication of nesfatin-1 and anxiolytic drugs.

Besides the correlation with self-reported anxiety, circulating NUCB2/nesfatin-1 was also associated with reported depression in a mixed-sex population [122], with NUCB2/nesfatin-1 levels rising with increasing severity of depression [123, 124], and correlated with inflammatory markers such as IL-6 and C-reactive protein as well as corticosterone under these conditions [125]. Higher NUCB2/nesfatin-1 levels were subsequently shown in patients reporting depression associated with subclinical hypothyroidism [126], leading to the hypothesis of nesfatin-1 being involved in these symptoms. Also, an animal model of gastric cancer comorbid with depression displayed higher NUCB2/nesfatin-1 peptide levels in the hippocampus, midbrain and plasma compared with undisturbed controls [127]. Nesfatin-1, at least based on animal data, is likely to play a role in the development of depressive symptoms, because ICV injection of the peptide was shown to reduce the consumption of a palatable snack in a novel environment as a surrogate for anhedonic behavior in rats [115]. This finding was subsequently extended to a peripheral effect as rats showed increased immobility in the forced swim test after IP injection of the peptide [116]. Whether nesfatin-1 plays a causal role in the development of depressive symptoms in humans as well remains to be investigated.

Interestingly, a positive association between circulating NUCB2/nesfatin-1 and depression has been described in women [118, 119], whereas in men this correlation was absent [120]. The

sex-specific alteration of the peptide was also described in a study on depression-associated suicide victims: while NUCB2 mRNA expression in the Edinger-Westphal nucleus was elevated in men, it was lower in women than in control subjects who died without any diagnosed neurodegenerative or psychiatric disorder [128]. This sex-specific regulation, possibly associated with a sex steroid dependency, as described below, should be followed up in future studies.

6. Implications of Nesfatin-1 in Cardiovascular Functions

ICV injection of nesfatin-1 was shown to increase blood pressure in rats [129, 130], an effect also observed after microinjection into the paraventricular nucleus of the hypothalamus [131] and probably mediated by increased central parasympathetic tone [132], assumed to mediate the observed bradycardia [133], and by sympathetic nerve outflow [134], and blocked by pretreatment with the melanocortin 3/4 antagonist SHU9119 [129, 130] or the α-adrenergic antagonist phentolamine [130]. Moreover, pretreatment with an oxytocin antagonist, ornithine vasotocin [22], or a CRF_2 antagonist, astressin$_2$-B [135], prevented nesfatin-1's hypertensive effect. Because the hypertensive action of CRF, but not α-melanocyte-stimulating hormone, was prevented by pretreatment with ornithine vasotocin [135], nesfatin-1 might act *via* downstream CRF → oxytocin → melanocortin 3/4 signaling to increase blood pressure. Also, microinjection of nesfatin-1 into the nucleus of the solitary tract increased blood pressure in rats [136]. Further corroborating the role of nesfatin-1 in the regulation of blood pressure, mice overexpressing NUCB2 displayed an increased systolic, diastolic, and mean blood pressure [137]. Conversely, knockdown of NUCB2 specifically in the paraventricular nucleus blunted the high-salt diet-stimulated increase in systolic blood pressure [21]. Likewise, silencing of paraventricular NUCB2/nesfatin-1 signaling counteracted the rise in systolic blood pressure observed in agouti-related peptide/3-phosphoinositide-dependent protein kinase-1 knockout mice [138].

Peripheral nesfatin-1 exerts pronounced effects on the cardiovascular system, namely an increase in blood pressure after IP administration in mice [139] and rats [140] or IV injection in rats [141], with inhibited relaxation of peripheral blood vessels probably contributing to this effect [141]. This effect is likely associated with stimulated signaling of the phosphoinositide-3-kinase (PI3K)/AKT/m-TOR pathway and phosphorylation of Janus kinase 2 (JAK2)/STAT3, resulting in proliferation, migration, and phenotype switch of vascular smooth muscle cells from a contractile to a synthetic state by elevating the mRNA and protein expression of matrix metalloproteinase 2 and 9 while reducing peroxisome proliferator-activated receptor-γ [142, 143]. Because NUCB2 mRNA expression was increased in the media of the aorta of spontaneously hypertensive rats [142], nesfatin-1 might play a pathogenetic role under these conditions. In line with this assumption, circulating NUCB2/nesfatin-1 levels were higher in patients with essential hypertension than in normotensive controls and correlated with systolic blood pressure [144]; therefore, nesfatin-1 has been suggested as a risk factor for obesity-associated hypertension (OR 1.5) [145]. Also, patients with polycystic ovary syndrome displayed increased circulating NUCB2/nesfatin-1 concentrations, which correlated with systolic and diastolic blood pressure levels [146].

In the heart nesfatin-1 decreases contractility and relaxation as assessed in isolated rat heart preparations [147]. In zebrafish, IP injected nesfatin-1 reduced end diastolic volume and cardiac output associated with decreased heart rate [148], whereas in rat [149] and goldfish [150] a positive inotropic effect was observed *ex vivo* [150], possibly reflecting the difference between whole body and organ or tissue conditions. These effects might well be locally mediated, because NUCB2 mRNA expression has been detected in the heart of mouse [14], rat [14], zebrafish [148], and human [14], a finding corroborated by NUCB2/nesfatin-1 protein expression in rat and human cardiomyocytes [14]. Because the receptor is assumed to be expressed in the heart, as recently suggested by autoradiography in rats [16], nesfatin-1 might act in the heart in an autocrine or paracrine manner.

The cardioprotective effects were first suggested after *ex vivo* experiments in Langendorff-perfused rat heart preparations, as indicated by reduced infarct size, lactate dehydrogenase

release and postischemic contracture [147]. These effects are probably mediated by myocardial upregulation of p-AKT/AKT and p-glycogen synthase kinase (GSK)-3beta/GSK-3β, resulting in a reduction of apoptotic and necrotic cells under conditions of isoproterenol-induced myocardial infarction [151]. Consequently, cardiac troponint and proinflammatory cytokines were lower in rats receiving IP nesfatin-1 than in those treated with vehicle [151]. However, another study reported a nesfatin-1-induced apoptosis of cardiomyocytes in isolated neonatal rat hearts mediated *via* reduced AKT inactivation and increased expression of apoptogenic protein 1 and caspase-3 [152]. Whether this discrepancy is caused by dosage differences (1 μM *vs.* 100 pM/L) warrants further investigation. Lastly, in a human study investigating subjects with rheumatoid arthritis, nesfatin-1 correlated positively with rheumatoid factor, matrix metalloproteinase-2, and plaque stability mediator and negatively with carotid intima-media thickness [153], giving rise to a protective effect of nesfatin-1 under these conditions.

7. Implications of Nesfatin-1 in Reproductive Functions

Expression of NUCB2 mRNA has been detected in rat [11], mouse [11], dog [154], and human [11] testes, and NUCB2/nesfatin-1 protein has been identified in the interstitium next to the seminiferous tubules [8], probably representing interstitial Leydig cells, with increased levels in the transition from puberty to adulthood stimulated by pituitary LH [11]. Similarly, in female mice NUCB2 mRNA expression was detected in the ovary, with NUCB2/nesfatin-1 peptide expression in the theca and interstitial cells of the ovary [13], the peptide expression has been confirmed in birds [155]. In female rats hypothalamic NUCB2 mRNA levels and NUCB2/nesfatin-1 protein content increased during pubertal transition [156]. Also, in humans circulating NUCB2/nesfatin-1 levels increased in girls with premature thelarche compared with the prepubertal state [157], leading to the hypothesis of a role for nesfatin-1 in gonadal development.

Indeed, ICV injection of nesfatin-1 resulted in an elevation of circulating gonadotropins, whereas ICV infusion of an anti-NUCB2 antisense oligonucleotide delayed vaginal opening and decreased ovarian weights as a sign of hampered puberty in these animals [156].

In male rats, nesfatin-1 increased human chorionic gonadotropin-stimulated testosterone secretion from testicular explants *ex vivo* [11]. ICV injection of nesfatin-1 resulted in a reduced expression of hypothalamic GnRH, kisspeptin, pituitary FSHβ, LHβ, and testicular steroidogenic acute regulatory protein, whereas it markedly elevated the expression of 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, and cytochrome P450 mRNA in the testes of pubertal rats [158]. Conversely, testosterone stimulated mouse NUCB2 mRNA and NUCB2/nesfatin-1 protein expression in hypothalamic and pituitary cells *in vitro* [159]. Further corroborating the testosterone-dependent regulation of nesfatin-1, castrated mice displayed reduced NUCB2 mRNA expression in the pituitary, a finding reversed by testosterone treatment [160].

Immunohistochemical expression analysis of hemochorial mouse and human placenta demonstrated a wide distribution of NUCB2/nesfatin-1, namely in the ectoplacental zone, parietal trophoblast giant cells and early spongiotrophoblast from embryonic day (E) 7.5 to E9.5 and additionally at E10.5 to E12.5 in the developing labyrinth [161]. Glycogen trophoblast cells, syncytiotrophoblast, sinusoidal trophoblast giant cells and fetal capillary endothelial cells of the labyrinth expressed NUCB2/nesfatin-1 at high levels from E12.5 [161]. In human pregnancy, nesfatin-1 was highly expressed in syncytiotrophoblast throughout all three trimesters [161], giving rise to a modulating role of nesfatin-1 in glucose homeostasis also during pregnancy. Nesfatin-1 was also recently implicated in the maintenance of pregnancy because uterine expression levels of NUCB2 mRNA and NUCB2/nesfatin-1 protein levels were increased in a mouse spontaneous abortion model [162], a hypothesis to be further investigated.

8. Conclusions

The present review highlights nesfatin-1 as a pleotropic polypeptide (Table 1) with a well-established anorexigenic action and an inhibitory effect on gastrointestinal motility acting on

Table 1. Central and Peripheral Effects of Nesfatin-1

	Central Effects	Mediation	Peripheral Effects	Mediation
Feeding behavior	Food intake ↓ (acute) [1, 21, 24], water intake ↑ [31]	CRF$_2$- [24] and histamine- [35], melanocortin- [1] dependent [35], pm-TOR-related [67]	Food intake ↓/= (acute and chronic) [2, 30]	Leptin-independent [2]
Gastrointestinal functions	Gastric emptying ↓ [24, 67] Gastric motility ↓ [69] Acid output ↓ [69]	Melanocortin receptor-dependent ↓ [67] H+/K+-ATPase expression ↓ [76]	Gastric contractions ↓ [73] Gastric ulcer healing ↑ [78–80]	Cyclooxygenase 2-dependent [79]
Glucose homeostasis and metabolism	Muscle glucose absorption, insulin receptor signaling ↑ [95], gluconeogenesis ↓ [95], hypothalamic glucosensing neurons ↑/↓ [34, 97], core body temperature ↑ [23], energy expenditure ↑ [110], dry heat ↓ [110], brown adipose tissue ↑ [110], tail temperature ↑ [110], body weight gain (chronic) ↓ [1, 34]	AKT/AMPK/transducer of regulated CREB protein 2 [95], melanocortin 3/4 signaling [110], UCP-1 expression and m-TOR signaling [112], melanocortin system, UCP1 expression [34]	Glucose-stimulated insulin secretion ↑ [90], glucagon secretion ↑ [85], insulin sensitivity ↑ [90], blood glucose ↓ [92], blood glucose and insulin resistance ↓ [93], brown adipocyte differentiation ↑ [112], UCP1 expression ↑ [112], plasma cholesterol + triacylglyceride ↓ [113], lipid accumulation ↓ [113], free fatty utilization ↑ [92]	K$_v$2.1 channel-dependent [88], AMPK and p-ACC phosphorylation [93], m-TOR signaling [112], AMPK phosphorylation [113]
Anxiety and depression	Anxiety and depressive-like behavior (acute) ↑ [115]		Anxiety-like behavior (chronic) ↑ [116]	
Cardiovascular functions	Blood pressure ↑ [130–132, 136], heart rate ↑/↓ [132], [133]	CRF- [22], sympathetic- [134], parasympathetic- [132], [133], melanocortin- [22] dependent	Blood pressure ↑ [139, 140], contractility of heart and vessels ↑ [141, 147], end diastolic volume, cardiac output, heart rate ↓ [148], aorta relaxation ↑ [149, 150], cardioprotection ↑ [147, 151], apoptosis of cardiomyocytes ↑/↓ [151, 152], proliferation, migration, phenotype switch of vascular smooth muscle cells ↑ [143]	PI3K/AKT/m-TOR, JAK2/STAT3 signaling [142], endothelium, NO- and guanylatecyclase-dependent [149], p-AKT/AKT and p-GSK-3β/GSK-3β [143], AKT, apoptogenic protein 1 and caspase-3 [152]. PI3K/AKT/m-TOR pathway and phosphorylation of JAK2/STAT3 [142]
Reproductive functions	Pubertal transition ↑ [156]	Circulating gonadotropins ↑ [156, 158], expression of genes for GnRH, kisspeptin, FSH, LH, testicular steroidogenic acute regulatory protein ↓ [158], expression of genes for 3β-HSD, 17β-HSD, cytochrome P450 ↑ [158]	Human chorionic gonadotropin-dependent testosterone secretion ↑ [158]	

Abbreviations: ↑, increase/stimulation; ↓, decrease/inhibition; =, no effect; NO, nitric oxide.
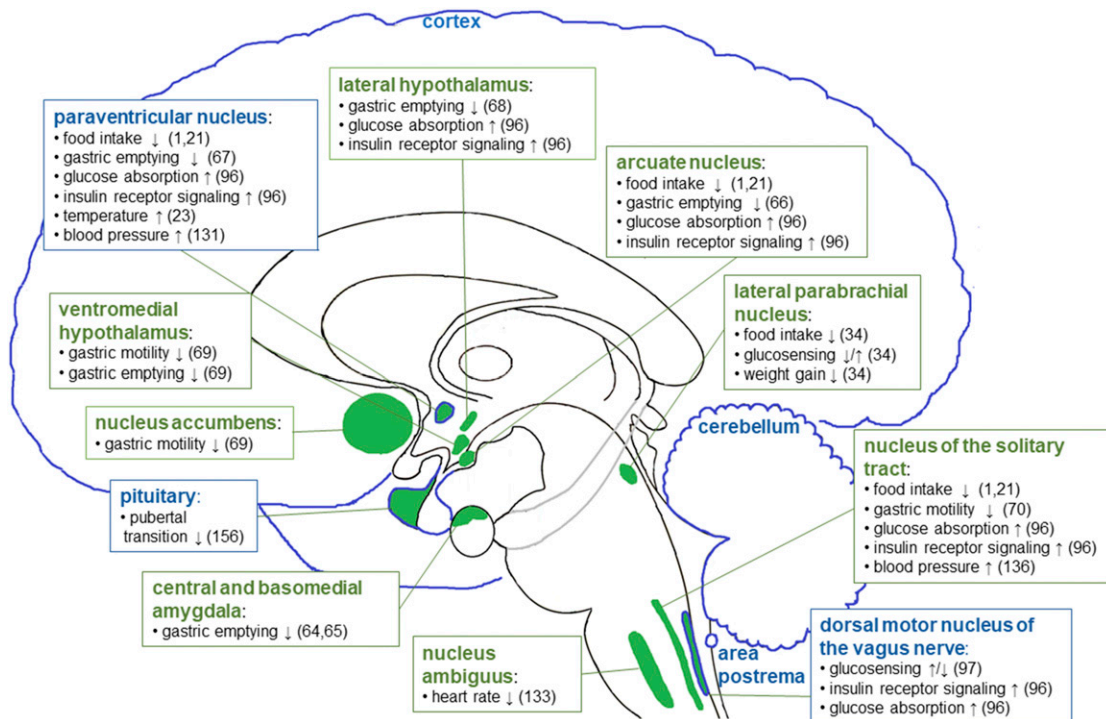
Figure 1. Central effects of nesfatin-1. Mediation of nesfatin-1's effects in different brain nuclei. The blue areas depict central nesfatin-1 binding sites as assessed by autoradiography, giving rise to the expression of the nesfatin-1 receptor [16]. ↑, increase/stimulation; ↓, decrease/inhibition.

different brain nuclei (Fig. 1). Moreover, nesfatin-1 plays a role in glucose homeostasis as a negative regulator of glucose levels and is also involved in energy expenditure by increasing thermogenesis. In addition, nesfatin-1 might be involved in the development of anxiety and depression as well, with a differential regulation in male and female patients highlighting the need for the study of sex differences. Its most striking peripheral effects are related to the cardiovascular system, thereby improving cardiac contractility, aorta relaxation, and cardioprotection. Lastly, peripheral nesfatin-1 is involved in gonadal development and during pregnancy. The identification of the yet unknown nesfatin-1 receptor will represent a great leap forward in the understanding of nesfatin-1's physiology and will allow us to better investigate the effects underlying the different actions summarized here.

Acknowledgments

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