Review

Hypothalamic Mechanisms Contributing to Cancer Cachexia

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Review

Hypothalamic Mechanisms Contributing to Cancer Cachexia

Hypothalamus and Cancer Cachexia

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Abstract: Cachexia is a complex multiorgan syndrome associated with various chronic diseases, characterized by anorexia and increased tissue wasting in the context of chronic inflammation. A specific form of this syndrome, known as cancer cachexia (CC), occurs alongside different types of tumors. The pathogenesis of CC is multifactorial, with inflammatory mediators and hormones released by either the tumor or the host identified as key drivers of the peripheral catabolic process through several direct mechanisms. Accumulating evidence indicates that the central nervous system (CNS) is also recognized as an integral component in the pathogenesis of CC. Hypothalamus has emerged as a critical brain area that senses and amplifies peripheral stimuli, generating an inappropriate neuronal signaling, leading to deregulation of feeding behavior and impaired control of energy homeostasis. Circulating cytokines may act in concert with hormones and neurotransmitters and perturbate the hypothalamic melanocortin system, shifting its activity towards the anorexigenic pathway and increase energy expenditure. The purpose of this review is to provide insights on the potential mechanisms mediating the hypothalamic inflammation in the context of anorexia and cachexia associated with cancer.

Keywords: cancer cachexia; anorexia; hypothalamus; cytokines; neurotransmitters; neuroendocrine

Introduction

Cachexia is a serious clinical condition associated with several illnesses including chronic kidney disease, cardiac failure, chronic obstructive pulmonary disease and cancer [1,2]. Cancer cachexia (CC) occurs in almost 80% of patients with advanced cancer and it is directly correlated with impaired quality of life and short survival [3]. The principal cachexia features include loss of appetite, inflammation, insulin resistance and skeletal muscle wasting, with or without fat mass loss [4]. The pathogenesis of this syndrome is complex and is gradually being unraveled. Inflammatory mediators and tumor derived factors have been proposed as direct triggers of the catabolic process in skeletal muscle and adipose tissue. Even though muscle and fat loss remain the principal features of CC, there is growing evidence that organs such as liver, heart and gut are also affected by cachexia factors.

The brain mediates several metabolic and behavioral aspects associated with CC by sensing and amplifying peripheral inflammatory signals and altering the pattern of neurotransmitters and neuropeptides involved in feeding. The hypothalamus is an important structure for appetite regulation and body energy expenditure and may play a crucial role in CC development. The mediobasal hypothalamus (MBH), which contains the ventromedial arcuate nucleus (ARC) and the media eminence (ME) complex, is a key region that lacks the blood brain barrier (BBB) and functions as a 'brain window' for sensing circulating inflammatory molecules and hormones [5]. In the ARC

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resides the melanocortin system (MCS), a critical neural system comprised of neurons with opposite effect on the control of appetite and the homeostatic feedback regulation of energy balance [6]. Upon peripheral stimuli, the MCS may respond inappropriately and disrupt the balance between orexigenic (appetite-stimulating) and anorexigenic (appetite-suppressing) pathways, leading to anorexia and hypercatabolic state [7]. In addition, it is known that hypothalamus is an important site for autonomic and endocrine homeostasis. Heightened sympathetic tone and aberrant neuroendocrine function have been observed in experimental cachexia models and are recognized as critical determinants of cancer associated tissue loss and increased energy wasting [8–12]. The paraventricular nucleus (PVN), specifically the parvocellular division, serves a crucial role in this process since it is able to integrate neuronal signals from hypothalamic nuclei and brainstem centers involved in appetite regulation (i.e., nucleus tractus solitarii-NTS and dorsal motor nucleus of the vagus-DMNV) and either generate a coordinated sympathetic output through projections to preganglionic neurons in the spinal cord [13] or secrete hypophysiotropic factors into the neurohemal zone of the median eminence modulating the neuroendocrine response [14].

The purpose of this review is to give information about cachexia mediators and neural pathways involved in central nervous system (CNS) dysfunction in the setting of cancer. The interplay between inflammatory molecules and neuronal groups will be discussed and how neuropeptides and neurohormones are affected to alter energy homeostasis and food intake. Although the process is complicated and involves multiple brain centers, this study will focus on the contribution of the hypothalamic mechanisms in driving this pathologic condition. Sheding light on the underlying pathophysiological pathways could help us identify novel molecular targets and expand the therapeutic options for cancer-related anorexia.

Cytokines Signaling in the Brain

Elevated circulating levels of pro-inflammatory cytokines have been reported in patients suffering from CC and have been associated with poor treatment outcomes [15–20]. These molecules have been shown to act on peripheral tissues stimulating catabolism and centrally affecting the mechanisms that regulate energy homeostasis [Figure 1]. Cytokines directly access the brain through the ME, where the BBB is incomplete, and reach the ARC. In addition, upon binding to their relative receptors on the BBB endothelial and glial cells can stimulate the synthesis of other chemical mediators such as prostaglandin-E2 (PGE2) and nitric oxide (NO) [21]. Finally, cytokines may communicate peripheral signals to brainstem centers via the vagal sensory terminals and ultimately to the hypothalamus [Figure 1] [22].

High cytokine levels have been detected in the hypothalami of tumor-bearing animals and associated with worsening of cancer associated anorexia [23–30]. Pharmacological activation of neuronal adenosine 5′-monophosphate-activated protein kinase (AMPK), a key component molecule involved in the regulation of energy homeostasis, was accompanied by the reduction of the hypothalamic levels of these cytokines, reversed anorexia and prolonged life span in animals [24].

Interleukin-1 (IL-1) is the prototype of the family of cytokines and in the brain plays a pivotal role in the organization of the hypothalamic cytokine network in response to systemic inflammatory challenges [31]. Under acute or chronic inflammatory stimuli the hypothalamic activity of IL-1β increases and correlates with the suppression of appetite [23,26–32]. In anorectic cancer animal models increased concentration of IL-1 in the cerebrospinal fluid (CSF) correlated inversely with food intake [33]. IL-1 interferes with the activity of the MCS as reported by studies using central administration of IL-1β and animal models of CC [34,35]. Selective treatment with intra-VMH injection of molecules that antagonize the IL-1 activity reduced the severity of cachexia [36].

Tumor-necrosis-factor-α (TNF-α) is another cytokine implicated in the induction of cachexia in acute and chronic diseases [37]. Rodents that received TNF-α both centrally and peripherally exhibited decrease in nutritional intake and wasting of body mass [38,39]. Its anorectic properties may be attributed to its suppressive action on chemosensitive neurons in the lateral hypothalamic area (LHA) and appetite regulating neurons in the ARC [40,41]. Moreover, increased hypothalamic TNF-α signaling has been shown to induce thermogenesis in the brown adipose tissue (BAT)

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mediated by an increase in β-adrenergic tone and rise of total body oxygen consumption, typical features of chronic cachectic states [42]. In experimental cancer models the use of recombinant human soluble TNF-α receptor or anti-TNF-α monoclonal antibodies improved food intake, body weight and increased survival [39,42].

The IL-6 family of cytokines is a group of cytokines that use the glycoprotein 130 (gp130) as the common signaling transducing component of the functional receptor complexes [43]. Among all the members of the IL-6 family, IL-6 and leukemia inhibitory factor (LIF) are considered to play a crucial role in the pathogenesis of anorexia and wasting syndrome in cancer. These molecules signal through activation of the janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, which is the same pathway induced by leptin [43]. IL-6 is produced by both immune and tumor cells [44]. IL-6 plays a prominent role in the anorexia of infection and increased brain production has been associated with the development of LPS induced sickness behavior [45]. Central delivery or overexpression of IL-6 in the CNS, dampened feeding and increased energy expenditure and tissue wasting [46–48]. It is likely that IL-6 exerts its modulatory effects on metabolism by acting at the hypothalamus and affecting the expression of neuropeptides implicated in the control of energy balance [46,47,49]. Increased IL-6 signaling in the VMH induced fatty acid oxidation in the skeletal muscle through stimulation of the α-adrenergic pathway [50]. LIF is a pleiotropic cytokine with paradoxically opposite effects on different cell types [44]. LIF is produced by multiple cancer cell lines and fosters tumor development, metastasis and peripheral tissue wasting [51–57]. LIF can reach the brain parenchyma from the blood by crossing the BBB [58] where interacts at the hypothalamic level with the appetite regulating neuropeptides and interferes also with leptin secretion promoting hypophagia [52,57,59,60]. LIF can induce a sustained inflammatory reaction and a long-term anorectic response, in contrast with IL-1 and TNF-α, whose anorectic effect is attenuated after continuous administration [37]. In animals implanted with cancer cells LIF expression correlated with early onset and greater severity of anorexia-cachexia [60]. It has been demonstrated that LIF associated cachexia occurs independently of IL-6 because rLIF administration in wild type (WT) mice lacking IL-6 provoked anorexia and weight loss [51].

Hypothalamic Inflammation

Previous experiments demonstrated that peripheral administration of immune challenges and cytokines increased c-fos expression (marker of neural activity) in critical brain areas involved in feeding and metabolism, like the MBH and PVN [61–63]. In these centers high density of cytokine receptors is observed, implying that cytokines target these hypothalamic centers to mediate anorexia and metabolic changes [64–66]. Cytokines can act in the brain through paracrine interactions inducing further their local production, independently of their concentration in plasma, thus maintaining and amplifying locally the inflammatory signaling [44,67]. In the hypothalamus an important source of cytokines seems to be the activated microglia [68]. Infectious agents have been shown to stimulate cytokine production by microglia, which in turn affect the appetite regulating neuronal networks via NF-κB signaling [27,69–71]. In tumor bearing animals CD11b-positive hypothalamic microglia showed high expression of m-RNA levels of IL-1β, IL-6 and TNF-α in the early stages of the disease and before the appearance of cachexia symptoms [12]. Neurons whose cell bodies are mainly located in the periventricular hypothalamic area are also able to synthesize and release cytokines [72–74]. Inflamed endothelial cells further contribute to the amplification of the inflammatory state by increasing the expression of genes involved in cell adhesion, cytokine signaling and extracellular matrix (ECM) remodelling, leading to altered permeability of the BBB [75]. Tanycytes (specialized glial cells), lined at the floor of the 3rd ventricle in the MBH, are stimulated by cytokines and induce the biosynthesis of PGE2 through activation of the nuclear factor-κB (NF-κB) pro-inflammatory signaling pathway [76]. Finally, oligodendrocytes and macrophages are also recruited and participate in the sustainment of the inflammatory response [75]. The pathophysiology of hypothalamic inflammation is illustrated in Figure 1.

Inflammatory Signals Across the Gut-Brain Axis

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A mechanism linking gut microbiota with their metabolites and exacerbation of the hypothalamic inflammatory status is currently being investigated. Damage of the intestinal mucosa is well documented in animals and patients with CC and may lead to transposition of bacterial components like lipopolysaccharide (LPS) to the systemic circulation [Figure 1] [77–79]. In animals with advanced cancer high LPS plasma levels correlated with deterioration of hypothalamic inflammation, microglia polarization and severe appetite and weight loss [12,80]. In vitro analysis showed that addition of LPS in cultured hypothalamic cells induced the release of IL-6 and this response was significantly amplified upon addition of tumor secretomes, indicating that LPS and tumor derived factors may act synergistically augmenting the inflammatory hypothalamic response [80]. It is well known that LPS induces neuroinflammation by interacting with the toll-like receptor 4 (TLR4), abundantly expressed in microglial cells, leading to *phosphorylation* and nuclear *translocation* of the NF-κB *p65 subunit and the transcription of genes encoding cytokines* [81–83]. Myeloid differentiation primary response gene 88 protein (MyD88) and TIR (Toll/interleukin-1 receptor)-domain-containing adaptor-inducing beta interferon (TRIF) are two key adaptor proteins for the TLR4-mediated downstream signaling [82,84]. Blockade of MyD88 signaling mitigated the hypothalamic inflammatory gene expression and significantly attenuated anorexia in pancreatic cancer models [28]. Likewise, TRIF deficient mice inoculated with tumor cells displayed down-regulated levels of cytokines and chemokines in the hypothalamus and improved anorexia and weight loss [27].

The Role of Hypothalamic Microglia

Several studies uncovered the contribution of glial cells in the control of energy metabolism in association with brain inflammation. Hypothalamic microglia were assigned a key role in the pathogenesis of acute sickness behavior and obesity [27,71,85,86]. In these conditions, pharmacological depletion of microglia has been reported to produce beneficial effects by attenuating hypothalamic inflammation and neuronal stress [71,85,86]. Few studies investigated the role of microglia in the development of CC. In a rodent model with implantation of the AH-130 ascites hepatoma cancer cell line, accumulation and activation of microglia were detected in both PVN and ARC where specifically increased sympathetic tone and induced the anorexigenic pathway activity [87]. Treatment with the microglia inhibitor minocycline suppressed microglia and neuronal activation and alleviated the clinical cachectic features [87]. In a murine model of pancreatic ductal adenocarcinoma (PDAC) microgliosis within the MBH was observed at an early stage of disease progression [88]. However, in this case pharmacologic microglia removal worsened cachexia indicators (anorexia, fatigue and muscle catabolism), underlying the potential protective function of microglia against PDAC induced cachexia [88]. Indeed, in vitro analysis showed that microglia responded to tumor derived factors by increasing the production of arginase-1, an enzyme with potent anti-inflammatory neuroprotective properties, and attenuating the production of IL-1 and TNF-α, both of which are implicated in neurotoxicity [88–90]. Even though the reason of these opposite results is not precisely understood, it is well accepted that microglia are highly heterogeneous cells and display distinguished phenotypic features when activated based on differences in the regional microenvironment. Moreover, in virtu of their plasticity these cells can reveal diversity of transcriptional profiles and functionality, depending on disease context and evolution [91]. Recent data reported the presence of two transcriptionally and functionally distinct subpopulations of activated microglia in the MBH of pancreatic cancer models with different gene expression signatures and cachexia-related responses [75]. The CD45(+) subset which expresses genes linked to a pro-inflammatory and neurotoxic profile and the CD45(-) subset which presents a transcriptional profile associated with neuronal growth, tissue remodeling and cell signaling [75]. These observations indicate that different clusters of disease related microglia coexist and at least one cluster appears to have a protective role in pancreatic cancer.

The Melanocortin System

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The ARC in rodents (infundibular nucleus in humans) is located in the MBH and its role is critical for the maintenance of energy homeostasis. Within the ARC resides the MCS composed of two distinct subpopulations of neurons with opposite effects on feeding and energy balance [6]. One cluster includes the pro-opiomelanocortin (POMC) and cocaine and amphetamine regulated transcript (CART) expressing neurons. POMC is a pro-hormone that is processed to biologically active peptides including the alpha-melanocyte-stimulating hormone (α-MSH), which act as an agonist at its specific subtypes melanocortin 4 receptors (MC4R) at the PVN to suppress food intake and increase energy expenditure [6]. Adjacent to POMC/CART neurons a second cluster of neurons express the orexigenic peptides Agouti-related peptide (AgRP) and neuropeptide Y (NPY) [6]. AgRP is the inverse agonist for MC3R and MC4R and antagonizes the effects of α-MSH. Neuropeptide Y (NPY) is a potent stimulator of food intake through its combined action on both the receptor subtypes Y1 and Y5 [92]. Under positive energy balance the expression of AgRP and NPY in the ARC is decreased while that of POMC and CART is enhanced. Conversely, in conditions of negative energy balance, the activity of the anorexigenic POMC/CART neurons is suppressed and the activity of NPY/AgRP neurons is promoted, resulting in reduction of the basal metabolism and increase in food consumption [7]. In the context of cancer wasting syndrome, the hypothalamus does not respond appropriately to neuroendocrine signals and the equilibrium between the neuropeptidergic circuits is dysregulated (Figure 2). Upregulated MC4R signaling was observed in the hypothalami of rodents with advanced cancer, anorexia and significant tissue loss, while central infusion of AgRP or other MC3/4-R antagonists ameliorated hypothalamic inflammation and cachexia [93–96]. This observation led to the hypothesis that interruption of the MC4R pathway may prove beneficial for the treatment of CC. Novel selective MC4R inhibitors with high bioavailability and BBB penetration capacity have been developed and when administered peripherally have been shown to protect against tumor-induced cachexia in animals [97,98].

Cytokines may directly interact and influence the activity of the MCS [Figure 2]. For example, receptors for IL-1 and LIF have been detected on POMC neurons [34,59]. In response to IL-1 administration POMC neurons increased their firing rate and the release of α-MSH from hypothalamic explants [34]. Centrally LIF-treated mice exhibited decreased food consumption associated with increased POMC activity and a-MSH secretion [59]. Conversely, IL-1 and TNF-α down-regulated the activity of AgRP-producing neurons and inhibited the release of AgRP [41].

Semaphorins of class 3 (SEMA3) are signaling secreted molecules with a regulatory role in tumor microenvironment and cancer cell behavior [99]. Semaphorin 3D (SEMA3D) has been shown to interact with its receptors neurophilin and plexin located on POMC neurons to drive the development of the hypothalamic circuit and energy balance [Figure 2] [100]. In a recent paper increased SEMA-3D expression was reported in patients and animals with CC which was positively related to increased hypothalamic POMC levels [101]. Mice with deficiency of SEMA3D showed decreased POMC neural activity and improvement of cachexia parameters [101].

The levels of hypothalamic POMC m-RNA have been found to be low, high or even unchanged among different cancer models or vary in the same model as the disease progresses [23,24,30,60,75,102–106]. It has been postulated that acute or chronic inflammatory stimuli may exert opposing signals on POMC expression. In conditions of acute inflammation POMC expression is induced, whereas in chronic inflammation POMC expression is suppressed. Administration of LPS (a typical model that resembles acute inflammation) in animals, induced an extensive cytokine response that promoted POMC expression, leading to an anorexic state and increased energy expenditure [107]. In the contest of malignancy, early (acute) or advanced (chronic) stage of the disease is associated with high or reduced POMC levels, respectively. Cernackova et al. found increased POMC transcript levels at day 21 in fibrosarcoma bearing Wistar rats, before cachexia was detected, and decreased POMC levels at day 28 when cachectic manifestations were remarkable [30]. Likewise, Suzuki et al. noted a pronounced decrease of POMC expression in hypothalamus of gastric cancer bearing rats only in advanced-stage cachexia with severe anorexia [105]. A previous report revealed that the *p65* subunit of the transcription factor NF-κB binds to the POMC promoter and activate its transcription in acute inflammatory conditions. However, in chronic inflammatory states

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due to increased methylation at and near the p65 binding site within the POMC promoter, the p65 subunit is unable to interact with the promoter, resulting in failure of the NF-κB-induced POMC transcription. At the same time p65 interacts with the phosphorylated s*ignal transducer and activator of transcription 3* (STAT3) preventing its binding to the POMC promoter, further repressing its transcriptional activation [108].

Lipocalin-2 (LCN2) was recently identified as an endogenous osteoblast-derived appetite-suppressing factor with central action [109]. Mosialou et al. showed that in normal mice when LCN2 was injected intraperitoneally was able to cross the BBB and inhibit food intake through a MC4R signaling dependent manner in neurons located in the PVN and VMH [Figure 2] [109]. Elevated hypothalamic and circulating levels of LCN2 have been detected in murine cancer models and associated with late cachexia stage [12,29,75,96]. Conversely, tumor bearing mice with genetic deletion of LCN2 showed improvement of energy intake and tissue wasting [75,96]. It has been reported that tumor cells stimulate the neutrophils of the bone marrow compartment to produce and secrete LCN2 and that this circulating LCN2 is able to penetrate the BBB and negatively regulate feeding behaviour [96]. Huisman et al. reported that in a pancreatic cancer murine model LCN2 was a strong stimulator of hypothalamic inflammation and affected gene expression in POMC neurons, but without affecting POMC expression itself [75]. Since anorexia in this model occurred independently of POMC expression and could be reversed with melanocortin antagonists, the authors concluded that LCN2 is likely to bind to and activate the MC4R, thus by-passing the physiologic melanocortin signaling pathway [96,97]. Further studies are warranted in order to determine the potential interaction between LCN2 and MC4R in pathologic conditions.

Neurotransmitters Implicated in Energy Balance

1. NPY

NPY acts mainly in the PVN and its release is associated with increased food intake. NPY secretion is stimulated in conditions of negative energy balance such as fasting, exercise and suppressed after refeeding [110]. In CC the NPYergic system seems to be dysfunctional [111]. Central administration of NPY does not elicit feeding response in tumor-bearing rodents compared to non-tumor bearing counterparts [112]. The levels of NPY m-RNA are either unaltered or increased in anorexic animals but without a compensatory increase in food intake [23,30,60,75,103,111,113–115]. Various hormonal and metabolic signals have been reported to affect the synthesis and secretion of NPY. Leptin and lactate decrease NPY m-RMA levels through AMPK inhibition, whereas ghrelin and glucocorticoids (GC) induces NPY gene expression through AMPK activation [116–119]. However, in contrast to elevated NPY transcript levels in hypothalamus, reduced protein levels are detected in the PVN, suggesting an impaired NPY transport and release at this specific region [120,121]. The increased serotonergic tone may contribute to this by interfering with NPY signaling at the post transcriptional level and repress its secretion [115]. Leptin has also been shown to suppress NPY secretion in hypothalamic cell lines [116].

2. 5-hydroxytryptamine

The neurotransmitter 5-hydroxytryptamine (5-HT) or serotonin has long been considered as a potent anorectic agent with central action. There is evidence that the serotonergic pathway originates from the raphe nucleus in the brainstem and lesions of these nuclei induce hyperphagia [122,123]. 5-HT neurons in the raphe nuclei project to the ARC where they regulate the neural activity of the MCS [122]. 5-HT leads to direct activation of the POMC neurons via interaction with 5-HT2c, and inhibition of the NPY/AgRP neurons via interaction with 5-HT1b subtype receptors [Figure 2] [124,125]. In experimental cancer systems elevated expression levels of 5-HT and HT1b have been observed in hypothalamic nuclei and correlated inversely with food consumption [126–129]. Tumor removal normalized these alterations and restored appetite [127,128]. Intrahypothalamic delivery of the non-selective 5-HT antagonist mianserin improved anorexia significantly in cachectic animals with cancer

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[130]. Cytokines may interfere with the 5-HT turnover or regulate the plasma pool of tryptophan, the precursor molecule of 5-HT [110]. IL-1 when injected peripherically reduced food intake and increased plasma tryptophan, suggesting an increase in brain 5-HT synthesis [131]. High plasma and hypothalamic levels of tryptophan and the metabolite 5-hydroxy indoleacetic acid (5-HIAA), marker of 5-HT release, have been reported in both tumor-bearing rats and anorectic cancer patients [132,133].

3. Orexins

Orexins (A and B) are hypothalamic neuropeptides involved in the regulation of multiple functions like sleep-wake cycle, autonomic function, feeding behavior and energy homeostasis [134]. Orexin-secreting neurons are placed mainly in the LHA/perifornical area (PFA) and are activated in response to NPY positive projections originating from ARC neurons [135]. They in turn, project and release their product to other hypothalamic centers, including PVN, ARC and NTS. Orexin neurons in the LHA sense and respond to peripheral circulating metabolic signals such as glucose, leptin and ghrelin [Figure 2] [134]. Central delivery of orexin-A in rats enhanced food intake, although continuous infusion did not produce substantial changes on feeding and body weight [136,137]. Prepro-orexin m-RNA levels are induced during periods of negative energy balance, such as periods of fasting [136,138]. There is evidence that LPS-induced hypothalamic inflammation disrupts the orexigenic signaling in the LHA and leads to reduced physical activity and hypophagia in animals [139]. In a similar manner, tumor-induced inflammation in anorectic rats was paralleled by a reduction in the number of orexin mRNA-expressing neurons, predominantly in the PFA [139]. The anorectic chemotherapeutic agent cisplatin has been associated with increased hypothalamic levels of proinflammatory cytokines and reduction of prepro-orexin m-RNA [86,140]. Direct delivery of orexin-A to the ARC enhanced significantly the feeding response and the use of neuropeptide Y1 receptor antagonist abolished this effect [140]. These findings indicate that orexin signaling in the ARC stimulates the feeding response via an NPY-dependent pathway.

4. Nesfatin-1

Nesfatin-1 is an 82-amino acid polypeptide that is cleaved from the precursor protein nucleobindin-2 (NUCB2) [141]. Nesfatin-1/NUCB2 was first detected in rat brains, in the PVN, ARC and NTS and latter in various peripheral tissues [141,142]. Rats with deficiency of Nesfatin-1/NUCB2 in the PVN increased feeding, fat accumulation and body weight [143]. Overexpression of Nesfatin-1/NUCB2 has been correlated with cancer invasion and metastatic potential [144,145]. Elevated Nesfatin-1/NUCB2 m-RNA levels in the PVN have been reported in tumor-bearing mice with anorexia implying a role for this molecule in the etiology of the syndrome [146]. Several mechanisms involved in the Nesfatin-1/NUCB2-induced anorexia have been proposed. Central administration of SHU9119, a melanocortin-3/4 receptor antagonist, abolished the anorexia induced by Nesfatin-1/NUCB2, suggesting a possible interaction with the MCS [147]. Increase of the oxytocinergic signaling from PVN neurons to POMC neurons in the NTS, interaction with the corticotropin releasing factor (CRF) receptor system and down-regulation of the NPY-expressing neurons in the ARC have also been reported [148–150]. Finally, Nesfatin-1/NUCB2 has been shown to evoke a sympatho-excitatory response through extracellular signal regulated kinase (ERK)-dependent signaling in PVN-CRF neurons and enhance fat lipolysis and fatty-acid oxidation in skeletal muscle in pathologic contexts other than CC [151,152].

Peripheral Hormone Signals: Leptin and Ghrelin

Leptin is a protein hormone derived from the adipocytes involved in satiety promotion and increase in energy expenditure [153]. Leptin signals in the cell by binding to its transmembrane receptors (Lep-R) and activating the JAK/STAT pathway [154]. Leptin interacts with both NPY/AgRP and POMC neurons in the ARC producing opposing effects, as well as with neurons expressing the MC4R in the PVN [Figure 2] [155,156]. Low leptin plasma levels have been reported among patients

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and animals with CC but without a concomitant compensatory stimulation of appetite or weight gain [104,157–159]. There is evidence that cytokines that share the same post receptor pathway with leptin, are able to upregulate the anorexigenic and down-regulate the orexigenic pathways by mimicking leptin action. In particular, members of the IL-6 family induce the phosphorylation of *STAT3*, *similar to leptin* and have been demonstrated to trigger anorexia-cachexia in cancer animal models, providing an explanation for the persistence of anorexia and body weight loss, in spite of decreased leptin concentration [51,160]. Treatment with molecules that inhibit JAK activity suppressed the anorexia associated with cancer in animals, inhibited lipolysis and improved survival and all these effects were accompanied by the reduction of STAT3 phosphorylation in hypothalamic and adipose tissues [51].

Ghrelin, is an endogenous stomach derived ligand with high affinity for the growth hormone (GH) secretagogue receptor (GHS-R) [161]. Ghrelin promotes weight gain by increasing appetite and decreasing energy expenditure [162]. Independently of its action in regulating the activity of the pituitary GH releasing neurons, ghrelin directly activates the orexigenic NPY/AgRP neurons which express the GHS-R [163,164]. Ghrelin is also able to inhibit POMC neurons indirectly through an increase of the inhibitory GABAergic tone originating from the NPY/AgRP neurons [165,166]. Additionally, ghrelin is able to act peripherically by stimulating the GHS-Rs located at the vagal afferent terminals which in turn transmit the signal to the hypothalamus [167]. Ghrelin serum levels are frequently found increased in cachectic patients with cancer and in anorectic tumor carrying animals. However due to an altered ghrelin signaling (called ghrelin resistance) appetite remains suppressed [Figure 2] [93,168–172]. It has been postulated that the anorexigenic proinflammatory cytokines can interfere with the appetite stimulatory effect induced by ghrelin [172]. Modulation of the orexigenic ghrelin signaling in anorectic tumor bearing rats due to hypothalamic interaction of 5-HT and CRF via the 5-HT2c has also been reported [10]. Defects at the receptor level might also influence the physiologic ghrelin signaling. In support of this hypothesis, reduced expression of the GHS-R has been detected in animals treated with the anorexigenic agent cisplatin and oral administration of 5-HT2cR antagonist, enhanced ghrelin sensitivity through up-regulation of the hypothalamic GHS-R levels and attenuated anorexia [173]. However, in tumor-bearing animals the levels of hypothalamic GHS-R were found to be either similar to control animals or elevated and normalized after ghrelin administration at high doses [171,172]. Moreover, when ghrelin was given to patients and animals with cancer, food intake was stimulated, suggesting that presumably further increase of plasma ghrelin levels by exogenous administration may overcome the endogenous encountered resistance to the peptide, resembling that way the treatment for insulin-resistant state in type II diabetes by peripheral insulin delivery [169,171,174,175]. Ghrelin or ghrelin mimetics in animals with CC, along with the positive impact on orexigenic peptides exert also an anti-inflammatory hypothalamic action as reported by the decrease of IL-1 receptor transcripts levels [175].

Neuroendocrine and Autonomic Regulation

The neuroendocrine system is regulated by hypothalamic neurons that send projections to the ME and secrete neurohormones into the portal hypophyseal system to excite cells in the anterior pituitary gland (adenohypophysis). Among these trophic factors, CRF is released by neurons located within the parvocellular zone of the PVN and stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH induces the adrenal cortex to produce GC (cortisol in humans and corticosterone in rodents) which in turn exerts a negative feed-back control suppressing the hypothalamic-pituitary-adrenal axis (HPA) [176].

It is well accepted that GCs play a crucial role in muscle wasting by regulating specific intracellular signaling pathways which stimulate protein catabolism and inhibit protein synthesis [177]. Rise in endogenous GC levels have been reported in both cancer patients and tumor bearing animals and correlated with the severity of cachexia [11,12,30,178–180]. In adenomatous polyposis coli (APC) cachectic mice a rise in corticosterone levels in serum and in skeletal muscle was associated with the induction of the transcription of GC-responsive genes that promote muscle protein

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degradation (called atrogenes) and down-regulate phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of the rapamycin (mTOR) anabolic pathway [11].

Although GCs act as downstream effectors of the HPA axis the upstream mechanism by which HPA is activated in cancer cachexia is unknown. CRF possess a strong hypophagic action and has assumed particular relevance in the setting of cancer [10,11,26,120]. In mice with terminal stage pancreatic cancer, severe muscle loss was associated with elevated hypothalamic CRF gene expression [26]. I.c.v. administration of a CRF antagonist in rats inoculated with AH-130 ascites hepatoma cells increased feeding, suggesting that the CRF system is activated in malignancy [10].

Cytokines such as IL-1, IL-6, TNF-α have been shown to directly stimulate the expression of the CRF gene in hypothalamic 4B cells [49,181,182]. Central administration of serum anti-CRF abolished the IL-1-induced anorexia in Wistar rats and depletion of endogenous IL-6 was associated with reduced production of CRF [49,183]. The brain derived neurotrophic factor (BDNF) is a molecule with a central role in energy control also able to stimulate the anorexigenic CRF system. Single or continuous i.c.v. delivery of BDNF upregulated the CRF m-RNA levels predominantly in the PVN and local infusion of the a-helical CRF receptor antagonist counteracted the appetite suppressive effect evoked by BDNF [184].

Ultrastructural analysis revealed neuronal intercommunications between 5-HT axons and CRF-containing neurons in the PVN, indicating that a possible interaction between the central serotonergic system and CRF synthesis may exist [185]. In physiologic murine models, 5-HT agonists enhanced c-fos immunoreactivity in CRF-containing neurons in the parvocellular zone and increased CRF m-RNA expression [186]. The administration of 5-HT2cR antagonists decreased hypothalamic CRF level and ameliorated anorexia and body weight loss in tumor-bearing rats [10].

CRF positive neurons in the PVN also express the MC4R [187]. Several studies investigated the potential involvement of these receptors in the CRF induced anorexia with disaccording results [188,189]. Selective blockage of the MC4-R in rats did not affect the CRF-induced inhibition of food intake suggesting that the anorectic effect of CRF is independent of signaling pathways involving the MC4R [188]. On the contrary, the appetite suppressive effect following delivery of a synthetic α-MSH was abolished when a CRF receptor antagonist was used [189]. Experiments in CRF knock-out mice revealed that CRF was involved in the early phase of the α-MSH induced anorexia but not in the late phase, implying that other endogenous ligands of the CRF family might be responsible for the late anorexic effect [189]. A schematic representation of the molecules involved in the activation of the HPA axis in CC is presented in Figure 3.

The parvocellular PVN provides an important sympathoexcitatory output through connections with brainstem centers and pre-ganglionic neurons in the spinal cord as well as with local intrahypothalamic neurocircuits. Activation of the PVN-sympathetic adipose circuit has been reported in chronic restraint stress [190]. Increased sympathetic drive is documented in humans and experimental models of tumor cachexia and it is associated with white adipose tissue browning, resulting in lipid mobilization and augmented non-shivering thermogenesis [Figure 3] [8,191].

BDNF signaling in the PVN plays a key role in the regulation of sympathetic activity and energy expenditure. Wang et al. showed that leptin signaling in the ARC is able to regulate sympathetic innervation of adipose tissue through a downstream BDNF-expressing population of neurons in the PVN [192]. These neurons are placed in the medial and posterior part of the PVN and project their axons to the sympathetic preganglionic neurons in the intermedio lateral column of the spinal cord. BDNF is released at this site where interacts with its corresponding tropomyosine receptor tyrosine kinase B (TrkB), thereby stimulating BAT thermogenesis [192,193]. In addition, BDNF- positive neurons in the PVN receive dopaminergic projections from neurons located in the Substantia Nigra (SN). Bilateral lesions at this level increased BDNF release in PVN which in turn increased catabolism and thermogenesis in BAT, underlying the importance of upstream brain nuclei in the modulation of BDNF activity and BAT metabolism [194]. Even though remarkable increase in BDNF hypothalamic expression has been detected in anorectic tumor bearing rats, studies investigating the activity of BDNF in the PVN in the setting of CC are missing [105].

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LIF receptors are also expressed in neurons in the PVN [52]. In several mouse allograft cancer models Xu et al. showed that LIF was able to trigger neuronal activation in several brain regions including PVN [Figure 3] and promote tumor progression through a mechanism involving sympathetic activation and peripheral immune cellular response [52]. Sympathetic ablation led to inhibition of tumor growth without affecting significantly the LIF-mediated neuronal activation of the PVN, suggesting that cancer affects upstream brain centers to modulate the sympathoexcitatory output [52]. However, besides the clear antitumor effect, the cachectic parameters or the effects on adipose tissue were not directly evaluated in the study and additional work investigating the correlation between LIF-induced sympathetic response and adipose tissue dysfunction is required.

Conclusions

CC is a multifactorial chronic condition related to abnormalities of the central physiological mechanisms that regulate food intake and energy balance. The hypothalamus has been recognized as an essential center that receives peripheral blood-borne signals and messages from other important brain structures which it transduces in perturbated neuronal signaling, abnormal neuroendocrine response and metabolic dysregulation. Hypothalamic inflammation has been shown to promote anorexia by triggering changes in orexigenic and anorexigenic neural circuits and induce energy deficit. Understanding the complex interrelationship between cytokines, tumor-derived factors and brain neurotransmitters and neuropeptides could not only shed light on the underlying pathophysiology of the disorder but also prove indispensable in unveiling the potential therapeutic interventions for the wasting syndrome associated with cancer. Disrupting neural signaling pathways and interfering pharmacologically with cytokine action might be therapeutically exploited in order to ameliorate the cachexia symptoms.

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References

1. S. von Haeling, M.S. Anker, S.D. Anker, Prevalence and clinical impact of cachexia in chronic illness in Europe, USA, and Japan: facts and numbers update 2016. J Cachexia Sarcopenia Muscle. **7,** 507-509 (2016)
2. M.J. Tisdale, Biology of cachexia. J. Natl. Cancer Inst. **89,** 1763-1773 (1997)
3. M.J. Tisdale, Cachexia in cancer patients. Nat. Rev. Cancer **2,** 862-871 (2002)
4. W.J. Evans, J.E. Morley, J. Argiles, C. Bales, V. Baracos, D. Guttridge, A. Jatoi, K. Kalantar-Zadeh, H. Lochs, G. Mantovani, D. Marks, W.E. Mitch, M. Muscaritoli, A. Najand, P. Ponikowski, F. Rossi Fanelli, M. Schambelan, A. Schols, M. Schuster, D. Thomas, R. Wolfe, S.D. Anker, Cachexia: a new definition. Clin. Nutr. **27,** 793–799 (2008)
5. R.D. Cone, M.A. Cowley, A.A. Butler, W. Fan, D.L. Marks, M.J. Low, The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. Int. J. Obesity **25,** S63–S67 (2001)
6. R.D. Cone, Anatomy and regulation of the central melanocortin system, Nat. Neurosci. **8,** 571–578 (2005)
7. A. Laviano, A. Inui, D.L. Marks, M.M. Meguid, C. Pichard, F. Rossi Fanelli, M. Seelander, Neural control of the anorexia-cachexia syndrome. Am. J. Physiol. Endocrinol. Metab. **295,** E1000-1008 (2008)
8. M. Petruzzelli, M. Schweiger, R. Schreiber, R. Campos-Olivas, M. Tsoli, J. Allen, M. Swarbrick, S. Rose-John, M. Rincon, G. Robertson, R. Zechner, E.F. Wagner, A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. Cell Metab. **20,** 433–447 (2014)

9. R. Suriben, M. Chen, J. Higbee, J. Oeffinger, R. Ventura, B. Li, K. Mondal, Z. Gao, D. Ayupova, P. Taskar, D. Li, S.R. Starck, H.H. Chen, M. McEntee, S.D. Katewa, V. Phung, M. Wang, A. Kekatpure, D. Lakshminarasimhan, A. White, A. Olland, R. Haldankar, M.J. Solloway, J.Y. Hsu, Y. Wang, J. Tang, D.A. Lindhout, B.B. Allan, Antibody-mediated inhibition of GDF15–GFRAL activity reverses cancer cachexia in mice. Nat. Med. **26,** 1264-1270 (2020)
10. N. Fujitsuka, A. Asakawa, Y. Uezono, K. Minami, T. Yamaguchi, A. Niijima, T. Yada, Y. Maejima, U. Sedbazar, T. Sakai, T. Hattori, Y. Kase, A. Inui, Potentiation of ghrelin signaling attenuates cancer anorexia-cachexia and prolongs survival. Transl. Psychiatry 1, e23 (2011)
11. A. Martin, J. Castells, V. Allibert, A. Emerit, C. Zolotoff, V. Cardot-Ruffino, Y.S. Gallot, B. Vernus, V. Chauvet, L. Bartholin, L. Schaeffer, A.C. Durieux, C. Hourdé, F.B. Favier, L. Mazelin, D. Freyssenet, Hypothalamic-pituitary-adrenal axis activation and glucocorticoid-responsive gene expression in skeletal muscle and liver of Apc mice. J. Cachexia Sarcopenia Muscle **13,** 1686-1703 (2022)
12. Y. Suda, K. Nakamura, F. Matsuyama, Y. Hamada, H. Makabe, M. Narita, Y. Nagumo, T. Mori, N. Kuzumaki, M. Narita, Peripheral-central network analysis of cancer cachexia status accompanied by the polarization of hypothalamic microglia with low expression of inhibitory immune checkpoint receptors. Mol. Brain 17, 20 (2024)
13. N. Nunn, M. Womack, C. Dart, R. Barrett-Jolley, Function and pharmacology of spinally-projecting sympathetic pre-autonomic neurones in the paraventricular nucleus of the hypothalamus. Curr. Neuropharmacol. **9,** 262-277. (2011)
14. A.G. Watts, S.E. Kanoski, G. Sanchez-Watts, W. Langhans, The physiological control of eating: signals, neurons, and networks. Physiol. Rev. 102, 689-813 (2022)
15. G. Mantovani, A. Macciò, L. Mura, E. Massa, M.C. Mudu, C. Mulas, M.R. Lusso, C. Madeddu, A.J. Dessì, Serum levels of leptin and proinflammatory cytokines in patients with advanced –stage cancer at different sites, Mol. Med. **78,** 554-561 (2000)
16. J. Pfitzenmaier, R. Vessella, C.S. Higano, J.L. Noteboom, D. Wallace Jr, E. Corey, Elevation of cytokine levels in cachectic patients with prostate carcinoma. Cancer **97,** 1211-1216 (2003)
17. M. Krzystek-Korpacka, M. Matusiewicz, D. Diakowska, K. Grabowski, K. Blachut, I. Kustrzeba-Wojcicka, T. Banas, Impact of weight loss on circulating IL-1, IL-6, IL-8, TNF-alpha, VEGF-A, VEGF-C and midkine in gastroesophageal cancer patients. Clin. Biochem. **40,** 1353-1360 (2007)
18. C. Scheede-Bergdahl, H.L. Watt, B. Trutschnigg, R.D. Kilgour, A. Haggarty, E. Lucar, A. Vigano, Is IL-6 the best pro-inflammatory biomarker of clinical outcomes of cancer cachexia? Clin. Nutr. **31,** 85-88 (2012)
19. L. Lerner, T.G. Hayes, N. Tao, B. Krieger, B. Feng, Z. Wu, R. Nicoletti, M.I. Chiu, J. Gyuris, J.M. Garcia, Plasma growth differentiation factor 15 is associated with weight loss and mortality in cancer patients. J. Cachexia Sarcopenia Muscle **6,** 317-324 (2015)
20. Y.C. Hou, C.J. Wang, Y.J. Chao, H.Y. Chen, H.C. Wang, H.L. Tung, J.T. Lin, Y.S. Shan, Elevated Serum Interleukin-8 Level Correlates with Cancer-Related Cachexia and Sarcopenia: An Indicator for Pancreatic Cancer Outcomes. J. Clin. Med. 7, 502 (2018)
21. W.A. Banks, J.L. Lynch, O. Tulin O. Price, in The Neuroimmunological Basis of Behavior and Mental Disorder, ed. by A. Siegel, S.S. Zalcman (Springer New York, 2009), p.3
22. G.R. Johnston, N.R. Webster, Cytokines and the immunomodulatory function of the vagus nerve. Br. J. Anaesth. **102,** 453-462 (2009)
23. C.R. Plata-Salamán, S.E. Ilyin, D. Gayle, Brain cytokine mRNAs in anorectic rats bearing prostate adenocarcinoma tumor cells. Am. J. Physiol. 275, R566-573 (1998)
24. E.R. Ropelle, J.R. Pauli, K.G. Zecchin, M. Ueno, C.T. de Souza, J. Morari, M.C. Faria, L.A. Velloso, M.J. Saad, J.B. Carvalheira, A central role for neuronal adenosine 5′-monophosphate-activated protein kinase in cancer-induced anorexia. Endocrinology **148,** 5220-5229 (2007)
25. F.S. Lira, A.S. Yamashita, J.C. Rosa, F.L. Tavares, E. Caperuto, L.C. Carnevali Jr, G.D. Pimentel, R.V. Santos, M.L. Batista Jr, A. Laviano, F. Rossi-Fanelli, M. Seelaende, Hypothalamic inflammation is reversed by endurance training in anorectic-cachectic rats. Nutr. Metab. (Lond) **8,** 60 (2011)
26. K.A. Michaelis, X. Zhu, K.G. Burfeind, S.M. Krasnow, P.R. Levasseur, T.K. Morgan, D.L. Marks, Establishment and characterization of a novel murine model of pancreatic cancer cachexia. J. Cachexia Sarcopenia Muscle **8,** 824–838. (2017)
27. K.G. Burfeind, X. Zhu, P.R. Levasseur, K.A. Michaelis, M.A. Norgard, D.L. Marks, TRIF is a key inflammatory mediator of acute sickness behavior and cancer cachexia. Brain Behav. Immun. **73,** 364–374 (2018)
28. X. Zhu, K.G. Burfeind, K.A. Michaelis, T.P. Braun, B. Olson, K.R. Pelz, T.K. Morgan, D.L. Marks, MyD88 signalling is critical in the development of pancreatic cancer cachexia. J. Cachexia Sarcopenia Muscle **10,** 378–390 (2019)
29. B. Olson, M.A. Norgard, P.R. Levasseur, X. Zhu, D.L. Marks, Physiologic and molecular characterization of a novel murine model of metastatic head and neck cancer cachexia. J. Cachexia Sarcopenia Muscle **12,** 1312-1332 (2021)

 doi:10.20944/preprints202411.1200.v2

30. A. Cernackova, A. Tillinger, J. Bizik, B. Mravec, L. Horvathova, Dynamics of cachexia-associated inflammatory changes in the brain accompanying intra-abdominal fibrosarcoma growth in Wistar rats. J. Neuroimmunol. **15,** 376 (2023)
31. S. Layé, G. Gheusi, S. Cremona, C. Combe, K. Kelley, R. Dantzer, P. Parnet, Endogenous brain IL-1 mediates LPS-induced anorexia and hypothalamic cytokine expression. Am. J. Physiol. Regul. Integr. Comp. Physiol. **279,** R93-98 (2000)
32. R.M. Bluthé, S. Layé, B. Michaud, C. Combe, R. Dantzer, P. Parnet, Role of interleukin-1beta and tumour necrosis factor-alpha in lipopolysaccharide-induced sickness behaviour: a study with interleukin-1 type I receptor-deficient mice. Eur. J. Neurosci. **12,** 4447-4456 (2000)
33. E.I. Opara, A. Laviano, M.M. Meguid, Correlation between food intake and cerebrospinal fluid interleukin 1 alpha in anorectic tumor-bearing rats. Nutrition 11, 678-679 (1995)
34. J.M. Scarlett, E.E. Jobst, P.J. Enriori, D.D. Bowe, A.K. Batra, W.F. Grant, M.A. Cowley, D.L. Marks, Regulation of central melanocortin signaling by interleukin-1 beta. Endocrinology **148,** 4217-4225 (2007)
35. J.M. Scarlett, X. Zhu, P.J. Enriori, D.D. Bowe, A.K. Batra, P.R. Levasseur, W.F. Grant, M.M. Meguid, M.A. Cowley, D.L. Marks, Regulation of agouti-related protein messenger ribonucleic acid transcription and peptide secretion by acute and chronic inflammation. Endocrinology **149,** 4837-4845 (2008)
36. A. Laviano, T. Renvyle, M.M. Meguid, Z.J. Yang, C. Cangiano, F. Rossi Fanelli, Relationship between interleukin-1 and cancer anorexia. Nutrition **11,** 680-683 (1995)
37. K.G. Burfeind, K.A. Michaelis, D.L. Marks, The central role of hypothalamic inflammation in the acute illness response and cachexia. Semin. Cell Dev. Biol. **54,** 42-52 (2016)
38. A.P. Arruda, M. Milanski, T. Romanatto, C. Solon, A. Coope, L.C. Alberici, W.T. Festuccia, S.M. Hirabara, E. Ropelle, R. Curi, J.B. Carvalheira, A.E. Vercesi, L.A. Velloso, Hypothalamic actions of tumor necrosis factor alpha provide the thermogenic core for the wastage syndrome in cachexia. Endocrinology **151,** 683–694 (2010)
39. G.F. Torelli, M.M. Meguid, L.L. Moldawer, C.K. Edwards 3rd, H.J. Kim, J.L. Carter, A. Laviano, F. Rossi Fanelli, Use of recombinant human soluble TNF receptor in anorectic tumor-bearing rats. Am. J. Physiol. **277,** R850-855 (1999)
40. C.R. Plata-Salamán, Y. Oomura, Y. Kai, Tumor necrosis factor and interleukin-1 beta: suppression of food intake by direct action in the central nervous system. Brain Res. **448,** 106-114 (1988)
41. F.M. Chaves, N.S. Mansano, R. Frazão, J. Donato Jr, Tumor Necrosis Factor α and Interleukin-1β Acutely Inhibit AgRP Neurons in the Arcuate Nucleus of the Hypothalamus. Int. J. Mol. Sci. **21,** 8928 (2020)
42. A.P. Arruda, M. Milanski, T. Romanatto, C. Solon, A. Coope, L.C. Alberici, W.T. Festuccia, S.M. Hirabara, E. Ropelle, R. Curi, J.B. Carvalheira, A.E. Vercesi, L.A. Velloso, Hypothalamic actions of tumor necrosis factor alpha provide the thermogenic core for the wastage syndrome in cachexia. Endocrinology **151,** 683-694 (2010)
43. P.C. Heinrich, I. Behrmann, G. Müller-Newen, F. Schaper, L. Graeve, Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem. J. **334,** 297-314 (1998)
44. A.J. Grossberg, J.M. Scarlett, D.L. Marks, Hypothalamic mechanisms in cachexia Physiol. Behav. **100,** 478-489 (2010)
45. M.D. Burton, N.L. Sparkman, R.W. Johnson, Inhibition of interleukin-6 trans-signaling in the brain facilitates recovery from lipopolysaccharide-induced sickness behavior. J. Neuroinflammation 8, 54 (2011)
46. K. Timper, J.L. Denson, S.M. Steculorum, C. Heilinger, L. Engström-Ruud, C.M. Wunderlich, S. Rose-John, F.T. Wunderlich, J.C. Brüning, IL-6 Improves Energy and Glucose Homeostasis in Obesity via Enhanced Central IL-6 trans-Signaling. Cell Rep. **19,** 267-280 (2017)
47. V.C. Bobbo, D.F. Engel, C.P. Jara, N.F. Mendes, R. Haddad-Tovolli, T.P. Prado, D. Sidarta-Oliveira, J. Morar, L.A. Velloso, E.P. Araujo, Interleukin-6 actions in the hypothalamus protects against obesity and is involved in the regulation of neurogenesis. J. Neuroinflammation **18,** 192 (2021)
48. K. Wallenius, V. Wallenius, D. Sunter, S.L. Dickson, J.Q. Jansson, Intracerebroventricular interleukin-6 treatment decreases body fat in rats. Biochem. Biophys. Res. Commun. 293, 560-565. (2022)
49. A. Benrick, E. Schéle, S.B. Pinnock, I. Wernstedt-Asterholm, S.L. Dickson, L. Karlsson-Lindahl, J.O. Jansson, Interleukin-6 gene knockout influences energy balance regulating peptides in the hypothalamic paraventricular and supraoptic nuclei. J. Neuroendocrinol. 21 620-628 (2009)
50. C.K. Katashima, T. de Oliveira Micheletti, R.R. Braga, R.S. Gaspar, L.J.E. Goeminne, A. Moura-Assis, B.M. Crisol, R.S. Brícola, V.R.R. Silva, C. de Oliveira Ramos, A.L. da Rocha, M.R. Tavares, F.M. Simabuco, V.A. Matheus, L. Buscaratti, H. Marques-Souza, P. Pazos, D. Gonzalez-Touceda, S. Tovar, M. Del Carmen García, J.C.R. Neto, R. Curi, S.M. Hirabara, P.C. Brum, P.O. Prada, L.P. de Moura, J.R. Pauli, A.S.R. da Silva, D.E. Cintra, L.A. Velloso, E.R. Ropelle, Evidence for a neuromuscular circuit involving hypothalamic interleukin-6 in the control of skeletal muscle metabolism. Sci. Adv. **8,** eabm7355 (2022)
51. G. Arora, A. Gupta, T. Guo, A. Gandhi, A. Laine, D. Williams, C. Ahn, P. Iyengar, R. Infante, JAK Inhibitors Suppress Cancer Cachexia-Associated Anorexia and Adipose Wasting in Mice. JCSM Rapid Commun. **3,** 115-128 (2020)

52. Q. Xu, Y. Cao, F. Kong, J. Liu, X. Chen, Y. Zhao, C.H. Lai, X. Zhou, H. Hu, W. Fu, J. Chen, J. Yang, Multiple cancer cell types release LIF and Gal3 to hijack neural signals. Cell Res. **34,** 345-354 (2024)
53. C. Di Giorgio, S. Marchianò, E. Marino, M. Biagioli, R. Roselli, M. Bordoni, R. Bellini, G. Urbani, A. Zampella, E. Distrutti, A. Donini, L. Graziosi, S. Fiorucci, Next-Generation Sequencing Analysis of Gastric Cancer Identifies the Leukemia Inhibitory Factor Receptor as a Driving Factor in Gastric Cancer Progression and as a Predictor of Poor Prognosis. Front. Oncol. **12,** 939969 (2022)
54. F. Zhang, Y. Yan, X. Cao, C. Guo, K. Wang, S. Lv, TGF-β-driven LIF expression influences neutrophil extracellular traps (NETs) and contributes to peritoneal metastasis in gastric cancer. Cell Death Dis. **15,** 218 (2024)
55. S.C. Kandarian, R.L. Nosacka, A.E. Delitto, A.R. Judge, S.M. Judge, J.D. Ganey, J.D. Moreira, R.W. Jackman, Tumour-derived leukaemia inhibitory factor is a major driver of cancer cachexia and morbidity in C26 tumour-bearing mice. J. Cachexia Sarcopenia Muscle **9,** 1109-1120 (2018)
56. X. Yang, J. Wang, C.Y. Chang, F. Zhou, J. Liu, H. Xu, M. Ibrahim, M. Gomez, G.L. Guo, H. Liu, W.X. Zong, F.E. Wondisford, X. Su, E. White, Z. Feng, W. Hu, Leukemia inhibitory factor suppresses hepatic de novo lipogenesis and induces cachexia in mice. Nat. Commun. **15,** 627 (2024)
57. G.K. Arora, A. Gupta, S. Narayanan, T. Guo, P. Iyengar, R.E. Infante, Cachexia-associated adipose loss induced by tumor-secreted leukemia inhibitory factor is counterbalanced by decreased leptin. JCI Insight **3,** e121221 (2018)
58. W. Pan, A.J. Kastin, J.M. Brennan, Saturable entry of leukemia inhibitory factor from blood to the central nervous system. J. Neuroimmunol. **106,** 172-180 (2000)
59. A.J._Grossberg, J.M. Scarlett, X. Zhu, D.D. Bowe, A.K. Batra, T.P. Braun, D.L. Marks, Arcuate nucleus proopiomelanocortin neurons mediate the acute anorectic actions of leukemia inhibitory factor via gp130. Endocrinology **151,** 606-616 (2010)
60. K. Terawaki, Y. Sawada, Y. Kashiwase, H. Hashimoto, M. Yoshimura, M. Suzuki, K. Miyano, Y. Sudo, S. Shiraishi, Y. Higami, K. Yanagihara, Y. Kase, Y. Ueta, Y. Uezono, New cancer cachexia rat model generated by implantation of a peritoneal dissemination-derived human stomach cancer cell line. Am. J. Physiol. Endocrinol. Metab. **306,** E373-387 (2014)
61. J.K. Elmquist, T.E. Scammell, C.D. Jacobsen, C.B. Saper, Distribution of Fos-like immunoreactivity in the rat brain following intravenous lipopolysaccharide administration. J. Comp. Neurol. **371,** 85–103 (1996)
62. S. Tolchard, A.S. Hare, D.J. Nutt, G. Clarke, TNF alpha mimics the endocrine but not the thermoregulatory responses of bacterial lipopolysaccharide (LPS): correlation with 83. FOS-expression in the brain. Neuropharmacology **35,** 243–248 (1996)
63. M. Herkenham, H.Y. Lee, R.A. Baker, Temporal and spatial patterns of c-fos mRNA induced by intravenous interleukin-1: a cascade of non-neuronal cellular activation at the blood-brain barrier. J. Comp. Neurol. **400,** 175–196 (1998)
64. A. Ericsson, C. Liu, R.P. Hart, P.E. Sawchenko, Type 1 interleukin-1 receptor in the rat brain: distribution, regulation, and relationship to sites of IL-1-induced cellular activation. J. Comp. Neurol. **361,** 681–698 (1995)
65. M. Utsuyama, K. Hirokawa, Differential expression of various cytokine receptors in the brain after stimulation with LPS in young and old mice. Exp. Gerontol. **37,** 411–420 (2002)
66. H. Yamakuni, M. Minami, M. Satoh, Localization of mRNA for leukemia inhibitory factor receptor in the adult rat brain. J. Neuroimmunol. **70,** 45–53 (1996)
67. S.K. Patra, S. Arora, Integrative role of neuropeptides and cytokines in cancer anorexia-cachexia syndrome. Clin. Chim. Acta **413,** 1025-1034 (2012)
68. S.J. Hopkins, N.J. Rothwell, Cytokines and the nervous system I: expression and recognition. Trends Neurosci. **18,** 83-88 (1995)
69. W.L. Reis, C.X. Yi, Y. Gao, M.H. Tschöp, J.E. Stern, Brain innate immunity regulates hypothalamic arcuate neuronal activity and feeding behavior. Endocrinology **156,** 1303–1315 (2015)
70. P.G. Jang, C. Namkoong, G.M. Kang, M.W. Hur, S.W. Kim, G.H. Kim, Y. Kang, M.J. Jeon, E.H. Kim, M.S. Lee, M. Karin, J.H. Baik, J.Y. Park, K.U. Lee, Y.B Kim, M.S. Kim, NF-κB activation in hypothalamic pro-opiomelanocortin neurons is essential in illness- and leptin-induced anorexia. J. Biol. Chem. 285, 9706–9715 (2010)
71. M. Valdearcos, J.D. Douglass, M.M. Robblee, M.D. Dorfman, D.R. Stifler, M.L. Bennett, I. Gerritse, R. Fasnacht, B.A. Barres, J.P. Thaler, S.K. Koliwad, Microglial inflammatory signaling orchestrates the hypothalamic immune response to dietary excess and mediates obesity susceptibility. Cell Metab. **26,** 185-197 (2017)
72. C.D. Breder, C.A. Dinarello, C.B. Saper, Interleukin- 1 immunoreactive innervation of the human hypothalamus. Science **240,** 321-324 (1988)
73. L. Acarin, B. Gonzalez, B. Castellano, Neuronal, astroglial and microglial cytokine expression after an excitotoxic lesion in the immature rat brain. Eur. J. Neurosci. 12, 3505–3520 (2000)
74. J.T. Dwarkasing, R.F. Witkamp, M.V. Boekschoten, M.C. Ter Laak, M.S. Heins, K. van Norren, Increased hypothalamic serotonin turnover in inflammation-induced anorexia. BMC Neurosci. 17, 26 (2016)
75. C. Huisman, M.A. Norgard, P.R. Levasseur, S.M. Krasnow, M.G.P. van der Wijst, B. Olson, D.L. Marks, Critical changes in hypothalamic gene networks in response to pancreatic cancer as found by single-cell RNA sequencing. Mol. Metab. 58, 101441 (2022)

 doi:10.20944/preprints202411.1200.v2

76. M. Böttcher, H. Müller-Fielitz, S.M. Sundaram, S. Gallet, V. Neve, K. Shionoya, A. Zager, N. Quan, X. Liu, R. Schmidt-Ullrich, R. Haenold, J. Wenzel, A. Blomqvist, D. Engblom, V. Prevot, M. Schwaninger, NF-κB signaling in tanycytes mediates inflammation-induced anorexia. Mol. Metab. **39,** 101022 (2020)
77. R.G.F. Costa, P.L. Caro, E.M. de Matos-Neto, J.D.C.C. Lima, K. Radloff, M.J. Alves, R.G. Camargo, A.F.M. Pessoa, E. Simoes, P. Gama, D.C. Cara, A.S.F. da Silva, W.O Pereira, L.F. Maximiano, P.S.M. de Alcântara, J.P. Otoch, G. Trinchieri, A. Laviano, M. Muscaritoli, M. Seelaender, Cancer cachexia induces morphological and inflammatory changes in the intestinal mucosa. J. Cachexia Sarcopenia Muscle 10, 1116-1127 (2019)
78. L.B. Bindels, A.M. Neyrinck, A. Loumaye, E. Catry, H. Walgrave, C. Cherbuy, S. Leclercq, M. Van Hul, H. Plovier, B. Pachikian, L.G. Bermúdez-Humarán, P. Langella, P.D. Cani, J.P. Thissen, N.M. Delzenne, Increased gut permeability in cancer cachexia: mechanisms and clinical relevance. Oncotarget 9, 18224-18238 (2018)
79. H. Liu, Y. Cheng, Y. Qu, G. Wu, Unraveling the gut microbiota and short-chain fatty acids characteristics and associations in a cancer cachexia mouse model. Microb. Pathog. 183, 106332 (2023)
80. X. Li, T. Holtrop, F.A.C. Jansen, B. Olson, P. Levasseur, X. Zhu, M. Poland, W. Schalwijk, R.F. Witkamp, D.L. Marks, K. van Norren, Lipopolysaccharide-induced hypothalamic inflammation in cancer cachexia-anorexia is amplified by tumour-derived prostaglandin E2. J. Cachexia Sarcopenia Muscle 13, 3014-3027 (2022)
81. P.A. Carpentier, D.S. Duncan, S.D. Miller, Glial toll-like receptor signaling in central nervous system infection and autoimmunity. Brain Behav. Immun. **22,** 140–147 (2008)
82. T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat. Immunol. **11,** 373–384 (2010)
83. T. Kawai, S. Akira, Signaling to NF-kappaB by Toll-like receptors. Trends Mol. Med. **13,** 460–469 (2007)
84. M. Yamamoto, S. Sato, H. Hemmi, K. Hoshino, T. Kaisho, H. Sanjo, O. Takeuchi, M. Sugiyama, M. Okabe, K. Takeda, S. Akira, Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. Science **301,** 640–643 (2003)
85. S. Jin, J.G. Kim, J.W. Park, M. Koch, T.L. Horvath, B.J. Lee, Hypothalamic TLR2 triggers sickness behavior via a microglia-neuronal axis. Sci. Rep. 6, 29424 (2016)
86. Y.T. Kim, B.S. Park, H.R. Yang, S. Yi, I.S. Nam-Goong, J.G. Kim, Exploring the potential hypothalamic role in mediating cisplatin-induced negative energy balance. Chem. Biol. Interact. 385, 110733 (2023)
87. S. Kashihara, K. Shinohara, S. Ikeda, H. Tsutsui, Microglia contribute to cancer cachexia through affecting PVN neurons and POMC neurons. FASEB J. 34, 1-1 Experimental Biology 2020 Meeting Abstracts
88. K.G. Burfeind, X. Zhu, M.A. Norgard, P.R. Levasseur, C. Huisman, K.A. Michaelis, B. Olson, D.L. Marks, Microglia in the hypothalamus respond to tumor-derived factors and are protective against cachexia during pancreatic cancer. Glia 68, 1479-1494 (2020)
89. C.I. Chang, J.C. Liao, L. Kuo, Arginase modulates nitric oxide production in activated macrophages. Am. J. Physiol. 274, H342–H348 (1998)
90. L. Ye, Y. Huang, L. Zhao, Y. Li, L. Sun, Y. Zhou, G. Qian, J.C. Zheng, IL-1β and TNF-α induce neurotoxicity through glutamate production: a potential role for neuronal glutaminase. J. Neurochem. 125, 897-908 (2013)
91. T. Masuda, R. Sankowski, O. Staszewski, M. Prinz, Microglia Heterogeneity in the Single-Cell Era. Cell. Rep. **30,** 1271-1281 (2020)
92. A.D. Nguyen, N.F. Mitchell, S. Lin, L. Macia, E. Yulyaningsih, P.A. Baldock, R.F. Enriquez, L. Zhang, Y.C. Shi, S. Zolotukhin, H. Herzog, A. Sainsbury, Y1 and Y5 Receptors Are Both Required for the Regulation of Food Intake and Energy Homeostasis in Mice. PLoS One 7, e40191 (2012)
93. B.E. Wisse, R.S. Frayo, M.W. Schwartz, D.E. Cummings, Reversal of cancer anorexia by blockade of central melanocortin receptors in rats. Endocrinology 142, 3292-301 (2001)
94. D.L. Marks, N. Ling, R.D. Cone, Role of the central melanocortin system in cachexia. Cancer Res. **61,** 1432-1438 (2001)
95. M.A. Joppa, K.R. Gogas, A.C. Foster, S. Markison, Central infusion of the melanocortin receptor antagonist agouti-related peptide (AgRP(83-132)) prevents cachexia-related symptoms induced by radiation and colon-26 tumors in mice. Peptides **28,** 636-642 (2007)
96. B. Olson, X. Zhu, M.A. Norgard, P.R. Levasseur, J.T. Butler, A. Buenafe, K.G. Burfeind, K.A. Michaelis, K.R. Pelz, H. Mendez, J. Edwards, S.M. Krasnow, A.J. Grossberg, D.L. Marks, Lipocalin 2 mediates appetite suppression during pancreatic cancer cachexia. Nat. Commun. **12,** 2057 (2021)
97. X. Zhu, M.F. Callahan, K.A. Gruber, M. Szumowski, D.L. Marks, Melanocortin-4 receptor antagonist TCMCB07 ameliorates cancer- and chronic kidney disease-associated cachexia. J. Clin. Invest. **130,** 4921-4934 (2020)
98. S.M. Axiak-Bechtel, S.B. Leach, J.R. Newton-Northup, R.J. Milner, S.A. Fox-Alvarez, L.I. Fagman, K.A. Young, D.J. Tate, Z.M. Wright, J.D. Chretin, J.W. Allen, S.K. Yoshimoto, K.A. Selting, B.K. Flesner, C.R. White, T. Mills, M. Aherne, P.J. Bergman, L. Qi, K.A. Gruber, M.F. Callahan, Safety of TCMCB07, a melanocortin-4 antagonist peptide, in dogs with naturally occurring cachexia. J. Vet. Intern. Med. **37,** 2344-2355 (2023)
99. C. Bica, A. Tirpe, A. Nutu, C. Ciocan, S. Chira, E.S. Gurzau, C. Braicu, I. Berindan-Neagoe, Emerging roles and mechanisms of semaphorins activity in cancer.Life Sci. **318,** 121499 (2023)

100. A.A. van der Klaauw, S. Croizier, E. Mendes de Oliveira, L.K.J. Stadler, S. Park, Y. Kong, M.C. Banton, P. Tandon, A.E. Hendricks, J.M. Keogh, S.E. Riley, S. Papadia, E. Henning, R. Bounds, E.G. Bochukova, V. Mistry, S. O'Rahilly, R. Simerly, J.E.N. Minchin, I. Barroso, E.Y. Jones, S.G. Bouret, I.S. Farooqi, Human Semaphorin 3 Variants Link Melanocortin Circuit Development and Energy Balance. Cell **176,** 729–742 (2019)
101. Y. Zhang, Q. Xi, M. Zhong, Y. Jiang, Q. Zhuang, Z. Ding, S. Tan, J. Wang, H. Liu, Z. Zhang, B. Zhou, G. Wu, Tumor-derived semaphorin 3D promoting cancer cachexia via regulating hypothalamic pro-opiomelanocortin neurons. FASEB J. 37, e22980 (2023)
102. B.E. Wisse, M.W. Schwartz, D.E. Cummings, Melanocortin Signaling and Anorexia in Chronic Disease States. Ann. N. Y. Acad. Sci. **994,** 275-281 (2003)
103. J.T. Dwarkasing, M. van Dijk, F.J. Dijk, M.V. Boekschoten, J. Faber, J.M. Argilès, A. Laviano, M. Müller, R.F. Witkamp, K. van Norren, Hypothalamic food intake regulation in a cancer-cachectic mouse model. J. Cachexia Sarcopenia Muscle **5,** 159-169 (2014)
104. H. Suzuki, H. Hashimoto, M. Kawasaki, M. Watanabe, H. Otsubo, T. Ishikura, H. Fujihara, H. Ohnishi, E. Onuma, H. Yamada-Okabe, Y. Takuwa, E. Ogata, T. Nakamura, Y. Ueta, Similar changes of hypothalamic feeding-regulating peptides mRNAs and plasma leptin levels in PTHrP-, LIF-secreting tumors-induced cachectic rats and adjuvant arthritic rats. Int. J. Cancer 128, 2215-2223 (2011)
105. M. Suzuki, M. Narita, M. Ashikawa, S. Furuta, M. Matoba, H. Sasaki, K. Yanagihara, K. Terawaki, T. Suzuki, Y. Uezono, Changes in the melanocortin receptors in the hypothalamus of a rat model of cancer cachexia. Synapse **66,** 747-751 (2012)
106. W.T. Chance, S. Sheriff, R. Dayal, A. Balasubramaniam, Refractory hypothalamic alpha-mSH satiety and AGRP feeding systems in rats bearing MCA sarcomas. Peptides **24,** 1909-1919 (2003)
107. V. Sergeyev, C. Broberger, T. Hökfel, Effect of LPS administration on the expression of POMC, NPY, galanin, CART and MCH mRNAs in the rat hypothalamus. Brain Res. Mol. Brain Res. 90, 93-100 (2001)
108. X. Shi, X. Wang, Q. Li, M. Su, E. Chew, E.T. Wong, Z. Lacza, G.K. Radda, V. Tergaonkar, W. Han, Nuclear factor κB (NF-κB) suppresses food intake and energy expenditure in mice by directly activating the Pomc promoter. Diabetologia 56, 925-936 (2013)
109. I. Mosialou, S. Shikhel, J.M. Liu, A. Maurizi, N. Luo, Z. He, Y. Huang, H. Zong, R.A. Friedman, J. Barasch, P. Lanzano, L. Deng, R.L. Leibel, M. Rubin, T. Nickolas, W. Chung, L.M. Zeltser, K.W. Williams, J.E. Pessin, S. Kousteni, Corrigendum: MC4R-dependent suppression of appetite by bone-derived lipocalin 2. Nature **546,** 440 (2017)
110. J.T. Dwarkasing, D.L. Marks, R.F. Witkamp, K. van Norren, Hypothalamic inflammation and food intake regulation during chronic illness. Peptides **77,** 60-66 (2016)
111. N. Nara-ashizawa, T. Tsukada, K. Maruyama, Y. Akiyama, N. Kajimura, K. Yamaguchi, Response of hypothalamic NPY mRNAs to a negative energy balance is less sensitive in cachectic mice bearing human tumor cells. Nutr. Cancer **41,** 111-118 (2001)
112. W.T. Chance, A. Balasubramaniam, H. Thompson, B. Mohapatra, J. Ramo, J.E. Fischer, Assessment of feeding response of tumor-bearing rats to hypothalamic injection and infusion of neuropeptide Y. Peptides **17,** 797-801 (1996)
113. N. Nara-ashizawa, T. Tsukada, K. Maruyama, Y. Akiyama, N. Kajimura, K. Nagasaki, T. Iwanaga, K. Yamaguchi, Hypothalamic appetite-regulating neuropeptide mRNA levels in cachectic nude mice bearing human tumor cells. **50,** 1213–1219 (2001)
114. W.T. Chance, C. Xiao, R. Dayal, S. Sheriff, Alteration of NPY and Y1 receptor in dorsomedial and ventromedial areas of hypothalamus in anorectic tumor-bearing rats. Peptides **28,** 295–301 (2007)
115. J.T. Dwarkasing, M.V. Boekschoten, J.M. Argilès, M. van Dijk, S. Busquets, F. Penna, M. Toledo, A. Laviano, R.F. Witkamp, K. van Norren, Differences in food intake of tumour-bearing cachectic mice are associated with hypothalamic serotonin signalling. J. Cachexia Sarcopenia Muscle 6, 84-94 (2015)
116. S.S. Dhillon, S.A. McFadden, J.A. Chalmers, M.L. Centeno, G.L. Kim, D.D. Belsham, Cellular Leptin Resistance Impairs the Leptin-Mediated Suppression of Neuropeptide Y Secretion in Hypothalamic Neurons. Endocrinology **152,** 4138-4147 (2011)
117. S.H. Cha, M.D. Lane, Central lactate metabolism suppresses food intake via the hypothalamic AMP kinase/malonyl-CoA signaling pathway. Biochem. Biophys Res Commun. 386, 212-216 (2009)
118. S.H. Lockie, R. Stark, M. Mequinion, S. Ch'ng, D. Kong, D.C. Spanswick, A.J. Lawrence, Z.B. Andrews, Glucose Availability Predicts the Feeding Response to Ghrelin in Male Mice, an Effect Dependent on AMPK in AgRP Neurons Endocrinology **159,** 3605-3614 (2018)
119. H. Shimizu, H. Arima, M. Watanabe, M. Goto, R. Banno, I. Sato, N. Ozaki, H. Nagasaki, Y. Oiso, Glucocorticoids increase neuropeptide Y and agouti-related peptide gene expression via adenosine monophosphate-activated protein kinase signaling in the arcuate nucleus of rats. Endocrinology **149,** 4544-4553 (2008)
120. H.D. McCarthy, P.E. McKibbin, A.V. Perkins, E.A. Linton, G. Williams, Alterations in hypothalamic NPY and CRF in anorexic tumor-bearing rats. Am. J. Physiol. **264,** E638–E643 (1993)

121. M.M. Meguid, E.J. Ramos, A. Laviano, M. Varma, T. Sato, C. Chen, Y. Qi, U.N. Das, Tumor anorexia: effects on neuropeptide Y and monoamines in paraventricular nucleus. Peptides **25,** 261–266 (2004)
122. J.P. Voigt, H. Fink, Serotonin controlling feeding and satiety. Behav. Brain Res. **277,** 14-31 (2015)
123. M.A. Geyer, A. Puerto, D.B. Menkes, D.S. Segal, A.J. Mandell, Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. Brain Res. **106,** 257–269 (1976)
124. L.K. Heisler, E.E. Jobst, G.M. Sutton, L. Zhou, E. Borok, Z. Thornton-Jones, H.Y. Liu, J.M. Zigman, N. Balthasar, T. Kishi, C.E. Lee, C.J. Aschkenasi, C.Y. Zhang, J. Yu, O. Boss, K.G. Mountjoy, P.G. Clifton, B.B. Lowell, J.M. Friedman, T. Horvath, A.A. Butler, J.K. Elmquist, M.A. Cowley, Serotonin reciprocally regulates melanocortin neurons to modulate food intake. *Neuron* **51,** 239–249 (2006)
125. Y. Xu, J.E. Jones, D. Kohno, K.W. Williams, C.E. Lee, M.J. Choi, J.G. Anderson, L.K. Heisler, J.M. Zigman, B.B. Lowell, J.K. Elmquist, HT2CRs Expressed by Pro-Opiomelanocortin Neurons Regulate Energy Homeostasis. Neuron **60,** 582-589 (2008)
126. V. Bláha, Z.J. Yang, M.M. Meguid, J.K. Chai, A. Oler, Z. Zadák, Ventromedial nucleus of hypothalamus is related to the development of cancer-induced anorexia: in vivo microdialysis study. Acta Medica (Hradec Kralove) **41,** 3-11 (1998)
127. E.J. Ramos, S. Suzuki, M.M. Meguid, A. Laviano, T. Sato, C. Chen, U. Das, Changes in hypothalamic neuropeptide Y and monoaminergic system in tumor-bearing rats: pre- and post-tumor resection and at death. Surgery **136,** 270-276 (2004)
128. I.G. Makarenko, M.M. Meguid, L. Gatto, C. Chen, E.J. Ramos, C.G. Goncalves, M.V. Ugrumov, Normalization of hypothalamic serotonin (5-HT 1B) receptor and NPY in cancer anorexia after tumor resection: an immunocytochemical study. Neurosci. Lett. **383,** 322-327 (2005)
129. I.G. Makarenko, M.M. Meguid, L. Gatto, C.G. Goncalves, E.J. Ramos, C. Chen, M.V. Ugrumov, Hypothalamic 5-HT1B-receptor changes in anorectic tumor bearing rats. Neurosci Lett. **376,** 71-75 (2005)
130. A. Laviano, J.R. Gleason, M.M. Meguid, Z.J. Yang, C. Cangiano, F. Rossi Fanelli, Effects of intra-VMN mianserin and IL-1ra on meal number in anorectic tumor-bearing rats. J. Investig. Med. **48,** 40-48 (2000)
131. M. Sato, A. Laviano, M.M. Meguid, C. Chen, F. Rossi-Fanelli, K. Hatakeyama, Involvement of plasma leptin, insulin and free tryptophan in cytokine-induced anorexia. *Clin. Nutr.* **22,** 139–146 (2003)
132. W.T. Chance, M.F. von Meyenfeldt, J.E. Fischer, Changes in brain amines associated with cancer anorexia. Neurosci. Biobehav. Rev. **7,** 471-479 (1983)
133. C. Cangiano, A. Cascino, F. Ceci, A. Laviano, M. Mulieri, M. Muscaritoli, F. Rossi-Fanelli, Plasma and CSF tryptophan in cancer anorexia. J. Neural Transm. Gen. Sect. **81,** 225-233 (1990)
134. J. Gatfield, C. Brisbare-Roch, F. Jenck, C. Boss, Orexin receptor antagonists: a new concept in CNS disorders? Chem. Med.Chem. 5, 1197-1214 (2010)
135. C.F. Elias, C.B. Saper, E. Maratos-Flier, N.A. Tritos, C. Lee, J. Kelly, J.B. Tatro, G.E. Hoffman, M.M. Ollmann, G.S. Barsh, T. Sakurai, M. Yanagisawa, J.K. Elmquist, Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. J. Comp. Neurol. **402,** 442–459 (1998)
136. T. Sakurai, A. Amemiya, M. Ishii, I. Matsuzaki, R.M. Chemelli, H. Tanaka, S.C. Williams, J.A. Richardson, G.P. Kozlowski, S. Wilson, J.R. Arch, R.E. Buckingham, A.C. Haynes, S.A. Carr, R.S. Annan, D.E. McNulty, W.S. Liu, J.A. Terrett, N.A. Elshourbagy, D.J. Bergsma, M. Yanagisawa, Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. Cell **92,** 573-585 (1998)
137. A. Yamanaka, T. Sakurai, T. Katsumoto, M. Yanagisawa, K. Goto, Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. Brain Res. 849, 248-252 (1999)
138. X.J. Cai, P.S. Widdowson, J. Harrold, S. Wilson, R.E. Buckingham, J.R. Arch, M. Tadayyon, J.C. Clapham, J. Wilding, G. Williams, Hypothalamic orexin expression: modulation by blood glucose and feeding. Diabetes 48, 2132-2137 (1999)
139. A.J. Grossberg, X. Zhu, G.M. Leinninger, P.R. Levasseur, T.P. Braun, M.G. Myers Jr, D.L. Marks, Inflammation-induced lethargy is mediated by suppression of orexin neuron activity. J. Neurosci. **31,** 11376–11386 (2011)
140. F. Guo, L. Xu, S. Gao, X. Sun, N. Zhang, Y. Gong, Effect of orexin-A in the arcuate nucleus on cisplatin-induced gastric side effects in rats. Neurosci. Res. **143,** 53-60 (2019)
141. S. Oh-I, H. Shimizu, T. Satoh, S. Okada, S. Adachi S, K. Inoue, H. Eguchi, M. Yamamoto, T. Imaki, K. Hashimoto, T. Tsuchiya, T. Monden, K. Horiguchi, M. Yamada, M. Mori, Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature **443,** 709-712 (2006)
142. S.K. Rupp, A. Stengel, Interactions between nesfatin-1 and the autonomic nervous system-an overview. Peptides **149,** 170719 (2022)
143. D. Stephan, N. Taege, R. Dore, J. Folberth, O. Jöhren, M. Schwaninger, H. Lehnert, C. Schulz, Knockdown of Endogenous Nucb2/Nesfatin-1 in the PVN Leads to Obese-Like Phenotype and Abolishes the Metformin- and Stress-Induced Thermogenic Response in Rats. Horm. Metab. Res. **54,** 768-779 (2022)
144. L. Ren, D. Bao, L. Wang, Q. Xu, Y. Xu, Z. Shi, Nucleobindin-2/nesfatin-1 enhances the cell proliferation, migration, invasion and epithelial-mesenchymal transition in gastric carcinoma. J. Cell. Mol. Med. 26, 4986-4994 (2022)

 doi:10.20944/preprints202411.1200.v2

145. S. Ning, C. Liu, K. Wang, Y. Cai, Z. Ning, M. Li, L. Zeng, NUCB2/Nesfatin-1 drives breast cancer metastasis through the up-regulation of cholesterol synthesis via the mTORC1 pathway. J. Transl. Med. 21, 362 (2023)
146. J.R. Burgos, B.M. Iresjö, U. Smedh, MCG101-induced cancer anorexia-cachexia features altered expression of hypothalamic Nucb2 and Cartpt and increased plasma levels of cocaine- and amphetamine-regulated transcript peptides. Oncol. Rep. 35, 2425-2430 (2016)
147. G.L.C. Yosten, W.K. Samson, Nesfatin-1 exerts cardiovascular effects in brain: possible interaction with the central melanocortin system. Am. J. Physiol. Regul. Integr. Comp. Physiol. **297,** R330–R336 (2009)
148. Y. Maejima, U. Sedbazar, S. Suyama, D. Kohno, E. Takano, N. Yoshida, M. Koike, Y. Uchiyama, K. Fujiwara, T. Yashiro, T.L. Horvath, M.O. Dietrich, S. Tanaka, K. Dezaki, S. Oh -I, K. Hashimoto, H. Shimizu, M. Nakata, M. Mori, T. Yada, Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. Cell Metab 10, 355–365 (2009)
149. A. Stengel, M. Goebel, L. Wang, J. Rivier, P. Kobelt, H. Moennikes, N.W.G. Lambrecht, Y. Tache, Central nesfatin-1 reduces dark-phase food intake and gastric emptying: differential role of corticotropin-releasing-factor 2 receptor. Endocrinology **150,** 4911–4919 (2009)
150. C.J. Price, W.K. Samson, A.V. Ferguson, Nesfatin-1 inhibits NPY neurons in the arcuate nucleus. Brain Res. 1230, 99-106 (2008)
151. M. Tanida, H. Gotoh, N. Yamamoto, M. Wang, Y. Kuda, Y. Kurata, M. Mori, T. Shibamoto, Hypothalamic Nesfatin-1 Stimulates Sympathetic Nerve Activity via Hypothalamic ERK Signaling Diabetes 64, 3725-3736 (2015)
152. Y. Liu, X. Chen, Y. Qu, L. Song, Q. Lin, M. Li, K. Su, Y. Li, J. Dong, Central nesfatin-1 activates lipid mobilization in adipose tissue and fatty acid oxidation in muscle via the sympathetic nervous system. Biofactors 46, 454-464 (2020)
153. J.M. Friedman, Modern science versus the stigma of obesity. *Nature Medicine* **10,** 563–569 (2004)
154. H. Baumann, K.K. Morella, D.W. White, M. Dembski, P.S. Bailon, H. Kim, C.F. Lai, L.A. Tartaglia, The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. Proc. Natl. Acad. Sci. U S A **93,** 8374-8378 (1996)
155. M.A. Cowley, J.L. Smart, M. Rubinstein, M.G Cerdán, S. Diano, T.L. Horvath, R.D. Cone, M.J. Low, Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature **411,** 480-484 (2001)
156. M. Ghamari-Langroudi, D. Srisai, R.D. Cone, Multinodal regulation of the arcuate/paraventricular nucleus circuit by leptin. Proc. Natl. Acad. Sci. U S A 108, 355-360 (2011)
157. J.P. Simons, A.M. Schols, L.A. Campfield, E.F. Wouters, W.H. Saris, Plasma concentration of total leptin and human lung-cancer-associated cachexia. Clin Sci (Lond) 93, 273-277 (1997)
158. J. Smiechowska, A. Utech, G. Taffet, T. Hayes, M. Marcelli, J.M. Garcia, Adipokines in patients with cancer anorexia and cachexia. J. Investig Med. **58,** 554–559 (2010)
159. C. Bing, S. Taylor, M.J. Tisdale, G. Williams, Cachexia in MAC16 adenocarcinoma: suppression of hunger despite normal regulation of leptin, insulin and hypothalamic neuropeptide Y. J. Neurochem. **79,** 1004-1112 (2001)
160. P.D. Lambert, K.D. Anderson, M.W. Sleeman, V. Wong, J. Tan, A. Hijarunguru, T.L. Corcoran, J.D. Murray, K.E. Thabet, G.D. Yancopoulos, S.J. Wiegand, Ciliary neurotrophic factor activates leptin-like pathways and reduces body fat, without cachexia or rebound weight gain, even in leptin-resistant obesity. Proc. Natl. Acad. Sci. U S A 98, 4652-4657 (2001)
161. M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, K. Kangawa, Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature **402,** 656-660 (1999)
162. M. Nakazato, N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa, S. Matsukura, A role for ghrelin in the central regulation of feeding. Nature **409,** 194-198 (2001)
163. M.G. Willesen, P. Kristensen, J. Romer, Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. Neuroendocrinology **70,** 306-316 (1999)
164. J. Kamegai, H. Tamura, T. Shimizu T, S. Ishii, H. Sugihara I. Wakabayashi, Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. Diabetes **50,** 2438-2443 (2001)
165. M.A. Cowley, R.G. Smith, S. Diano S. M. Tschöp, N. Pronchuk, K.L. Grove, C.J. Strasburger, M. Bidlingmaier, M. Esterman, M.L. Heiman, L.M. Garcia-Segura, E.A. Nillni, P. Mendez, M.J. Low, P. Sotonyi, J.M. Friedman, H. Liu, S. Pinto, W.F. Colmers, R.D. Cone, T.L. Horvath, The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron **37,** 649–661 (2003)
166. S.R. Chen, H. Chen, J.J. Zhou, G. Pradhan, Y. Sun, H.L. Pan, D.P. Li, Ghrelin receptors mediate ghrelin-induced excitation of agouti-related protein/neuropeptide Y but not pro-opiomelanocortin neurons. J. Neurochem. 142, 512-520 (2017)
167. Y. Date, N. Murakami, K. Toshinai, S. Matsukura, A. Niijima, H. Matsuo, K. Kangawa, M. Nakazato, The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology 123, 1120-1128 (2002)

168. Y. Shimizu, N. Nagaya, T. Isobe, M. Imazu, H. Okumura, H. Hosoda, M. Kojima, K. Kangawa, N. Kohno, Increased plasma ghrelin level in lung cancer cachexia. Clin. Cancer Res. 9, 774-778 (2003)
169. J.M. Garcia, M. Garcia-Touza, R.A. Hijazi, G. Taffet, D. Epner, D. Mann, R.G. Smith, G.R. Cunningham, M. Marcelli, Active ghrelin levels and active to total ghrelin ratio in cancer-induced cachexia. J. Clin. Endocrinol. Metab. **90,** 2920–2926 (2005)
170. D. Murata, K. Azuma, N. Matsuo, K. Murotani, G. Matama, A. Kawahara, T. Sasada, T. Tokito, T. Hoshino, Survival and biomarkers for cachexia in non-small cell lung cancer receiving immune checkpoint inhibitors. Cancer Med. **12,** 19471-19479 (2023)
171. W. Wang, M. Andersson, B.M. Iresjo, C. Lonnroth, K. Lundholm, Effects of ghrelin on anorexia in tumor-bearing mice with eicosanoid-related cachexia. Int. J. Oncol. **28,** 1393–1400 (2006)
172. K. Terawaki, Y. Kashiwase, Y. Sawada, H. Hashimoto, M. Yoshimura, K. Ohbuchi, Y. Sudo, M. Suzuki, K. Miyano, S. Shiraishi, Y. Higami, K. Yanagihara, T. Hattori, Y Kase, Y. Ueta, Y Uezono, Development of ghrelin resistance in a cancer cachexia rat model using human gastric cancer-derived 85As2 cells and the palliative effects of the Kampo medicine rikkunshito on the model. PLoS One 12, e0173113 (2017)
173. K. Yakabi, S. Kurosawa, M. Tamai, M. Yuzurihara, M. Nahata, S. Ohno, S. Ro, S. Kato, T. Aoyama, T. Sakurada, H. Takabayashi, T. Hattori, Rikkunshito and 5-HT2C receptor antagonist improve cisplatin-induced anorexia via hypothalamic ghrelin interaction. Regul Pept. **161,** 97–105 (2010)
174. H. Tsubouchi, S. Yanagi, A. Miura, N. Matsumoto, K. Kangawa, M. Nakazato, Ghrelin relieves cancer cachexia associated with the development of lung adenocarcinoma in mice. Eur. J. Pharmacol. **743,** 1-10 (2014)
175. M.D. DeBoer, X.X. Zhu, P. Levasseur, M.M. Meguid, S. Suzuki, A. Inui, J.E. Taylor, H.A. Halem, J.Z. Dong, R. Datta, M.D. Culler, D.L. Marks, Ghrelin treatment causes increased food intake and retention of lean body mass in a rat model of cancer cachexia. Endocrinology 148, 3004-3012 (2007)
176. L.J. Rohrbasser, H. Alsaffar, J. Blair, in Principles of Endocrinology and Hormone Action, ed. by A. Belfiore, D. LeRoith (Springer, Cham, Switzerland, 2018) P.287
177. A.L. Goldberg, Protein turnover in skeletal muscle. II. Effects of denervation and cortisone on protein catabolism in skeletal muscle. J. Biol. Chem. **244,** 3223–3229 (1969)
178. M.P. Cala, M.T. Agullo-Ortuno, E. Prieto-Garcia, C. Gonzalez-Riano, L. Parrilla-Rubio, C. Barbas, C.V. Díaz-García, A. García, C. Pernaut, J. Adeva, M.C. Riesco, F.J. Rupérez, J.A. Lopez-Martin, Multiplatform plasma fingerprinting in cancer cachexia: a pilot observational and translational study. J. Cachexia Sarcopenia Muscle **9,** 348-357 (2018)
179. S.T. Russell, M.J. Tisdale, The role of glucocorticoids in the induction of zinc-alpha2 glycoprotein expression in adipose tissue in cancer cachexia. Br. J. Cancer **92,** 876-881 (2005)
180. P. Costelli, N. Carbo, L. Tessitore, G.J. Bagby, F.J. Lopez-Soriano, J.M. Argiles, F.M. Baccino, Tumor necrosis factor-alpha mediates changes in tissue protein turnover in a rat cancer cachexia model. J. Clin. Invest. **92,** 2783–2789 (1993)
181. K. Kageyama, S. Kagaya, S. Takayasu, K. Hanada, Y. Iwasaki, T. Suda, Cytokines induce NF-κB, Nurr1 and corticotropin-releasing factor gene transcription in hypothalamic 4B cells. Neuroimmunomodulation **17,** 305-313 (2010)
182. M. Tasso, K. Kageyama, Y. Iwasaki, Y. Watanuki, K. Niioka, S. Takayasu, M. Daimon, Growth differentiation factor-15 stimulates the synthesis of corticotropin-releasing factor in hypothalamic 4B cells. Peptides 170, 171112 (2023)
183. A. Uehara, C. Sekiya, Y. Takasugi, M. Namiki, A. Arimura, Anorexia induced by interleukin 1: involvement of corticotropin-releasing factor. Am. J. Physiol. 257, R613-617 (1989)
184. K. Gotoh, T. Masaki, S. Chiba, H. Ando, K. Fujiwara, T. Shimasaki, K. Mitsutomi, I. Katsuragi, T. Kakuma, T. Sakata, H. Yoshimatsu, Brain-derived neurotrophic factor, corticotropin-releasing factor, and hypothalamic neuronal histamine interact to regulate feeding behavior. J. Neurochem. 125, 588-598 (2013)
185. Z. Liposits, C. Phelix, W.K. Paull, Synaptic interaction of serotonergic axons and corticotropin releasing factor (CRF) synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. A light and electron microscopic immunocytochemical study. Histochemistry 86, 541-549 (1987)
186. L.D. Van de Kar, A. Javed, Y. Zhang, F. Serres, D.K. Raap, T.S. Gray, 5-HT2A receptors stimulate ACTH, corticosterone, oxytocin, renin, and prolactin release and activate hypothalamic CRF and oxytocin-expressing cells. J. Neurosci. **21,** 3572-3579 (2001)
187. X.Y. Lu, G.S. Barsh, H. Akil, S.J. Watson, Interaction between α-melanocyte-stimulating hormone and corticotropin-releasing hormone in the regulation of feeding and hypothalamo-pituitary-adrenal responses. J. Neurosci. **23,** 7863–7872 (2003)
188. A.V. Vergoni, A. Bertolini, J.E. Wikberg, H.B. Schioth Corticotropin-releasing factor (CRF) induced anorexia is not influenced by a melanocortin 4 receptor blockage. Peptides **20,** 509–513 (1999)
189. S. Kawashima, S. Sakihara, K. Kageyama, T. Nigawara, T. Suda, Corti cotropin-releasing factor (CRF) is involved in the acute anorexic effect of α-melanocyte-stimulating hormone: A study using CRF-deficient mice. Peptides **29,** 2169-2174 (2008)

190. J. Wang, L. Sun, J. You, H. Peng, H. Yan, J. Wang, F. Sun, M. Cui, S. Wang, Z. Zhang, X. Fan, D. Liu, C. Liu, C. Qiu, C. Chen, Z. Xu, J. Chen, W. Li, B. Liu, Role and mechanism of PVN-sympathetic-adipose circuit in depression and insulin resistance induced by chronic stress. EMBO Rep. **24,** e57176 (2023)
191. M. Erdem, D. Möckel, S. Jumpertz, C. John, A. Fragoulis, I. Rudolph, J. Wulfmeier, J. Springer, H. Horn, M. Koch, G. Lurje, T. Lammers, S. Olde Damink, G. van der Kroft, F. Gremse, T. Cramer, Macrophages protect against loss of adipose tissue during cancer cachexia J. Cachexia Sarcopenia Muscle **10,** 1128-1142 (2019)
192. P. Wang, K.H. Loh, M. Wu, D.A. Morgan, M. Schneeberger, X. Yu, J. Chi, C. Kosse, D. Kim, K. Rahmouni, P. Cohen, J. Friedman, A leptin-BDNF pathway regulating sympathetic innervation of adipose tissue. Nature **583,** 839-844 (2020)
193. J.J. An, G.Y. Liao, C.E. Kinney, N. Sahibzada, B. Xu, Discrete BDNF neurons in the paraventricular hypothalamus control feeding and energy expenditure. Cell Metab. **22,** 175–188 (2015)
194. Y. Zhang, L. Zhou, H. Lian, Y. Zhang, S. Tong, Z. Wang, Dopamine receptor 2 downregulation and brain-derived neurotrophic factor upregulation in the paraventricular nucleus are correlated with brown adipose tissue thermogenesis in rats with bilateral substantia nigra lesions. J. Chem. Neuroanat. **117,** 102016 (2021)