PHYSIOLOGICAL REVIEW

Ghrelin and its interactions with growth hormone, leptin and orexins: Implications for the sleep–wake cycle and metabolism

Fabio García-García [a,b,*], Enrique Juárez-Aguilar [b], Juan Santiago-García [c], Daniel P. Cardinali [d]

[a] Laboratorio de Biología de Sueño, Departamento de Biomedicina, Instituto de Ciencias de la Salud, Universidad Veracruzana, Mexico
[b] Departamento de Biomedicina, Instituto de Ciencias de la Salud, Universidad Veracruzana, Mexico
[c] Instituto de Investigaciones Biológicas, Universidad Veracruzana, Mexico
[d] Departamento de Docencia e Investigación, Pontificia Universidad Católica Argentina, Buenos Aires, Argentina

SUMMARY

Several studies have shown that ghrelin administration promotes wakefulness in rodents, while in human males it induces sleep but has no effect in women. Ghrelin also plays an important role in metabolism and appetite regulation, and as described in this review may participate in the energy balance during sleep. In this review, we summarize some of the effects induced by ghrelin administration on the sleep–wake cycle in relation to the effects of other hormones, such as growth hormone, leptin, and orexin. Finally we discuss the relationship between sleep deprivation, obesity and ghrelin secretion pattern.

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Introduction

Ghrelin's role in appetite and energy balance regulation is well documented. In recent years new evidence has emerged demonstrating the role of ghrelin in the sleep–wake cycle, both in animals and humans. This topic has been addressed in two excellent reviews published by the group of A. Steiger.[1,2] However, to our knowledge there are no reviews addressing the role of ghrelin regulating both behaviors. In this review we summarize some of the effects induced by ghrelin administration on the sleep–wake cycle in experimental animals and humans. We also discuss the possible interactions of ghrelin with growth hormone, leptin, and orexins in the regulation of the sleep–feeding circuit, emphasizing ghrelin's potential role on the energy balance during sleep. Finally, we address how the lack of sleep could be a trigger for the development of obesity and whether ghrelin is part of it.

Ghrelin is a 28 amino acid peptide secreted mainly by the stomach and is an endogenous ligand for the growth hormone secretagogue receptor 1a (GHS-R1a).[3] GHS-R1a is a G protein-coupled receptor widely expressed in peripheral tissues, as well as in various brain regions, such as the hypothalamus, thalamus, cortex, hippocampus and the pituitary gland.[4,5] The hypothalamus is the main brain region of ghrelin synthesis,[6] although overall peptide brain levels are much lower than those found in the stomach.

Ghrelin is derived from a preprohormone called preproghrelin, which generates, by post-translational cleavage, a second peptide of 26 amino acid called obestatin,[7] and a third peptide of 60 amino acids, called C-ghrelin (reviewed by Seim et al.[8]). In addition, the primary mRNA encoded by the ghrelin gene can also generate multiple transcripts by alternative splicing, some of them may encode peptides of unknown function.[8] Ghrelin is involved in growth hormone release, metabolism and appetite regulation (reviewed by Chen et al.[9]), as well as in the sleep–wake cycle regulation as described[1,2] and in this review. Obestatin was initially reported as a ligand for the orphan G protein-coupled receptor GPR39, involved in satiety and decreased food intake[7]; however there is controversy on these findings, and the role of this peptide is not well established (reviewed by Seim et al.[10]). Obestatin also induces sleep when centrally administered to rats.[11] On the other hand, C-ghrelin circulates at high levels in plasma; however, its function and putative receptor are unknown.[8]

* Corresponding author. Departamento de Biomedicina, Instituto de Ciencias de la Salud, Universidad Veracruzana, 91190 Xalapa, Veracruz, Mexico. Tel.: +52 228 8418925; fax: +52 228 8418926.
E-mail address: fgarcia@uv.mx (F. García-García).

Abbreviations			
AgRP	agouti-related peptide	LDTg	laterodorsal tegmental nucleus
ARC	arcuate nucleus of the hypothalamus	LHA	lateral hypothalamic area
BMI	body mass index	MPA	medial preoptic area
CART	cocaine and amphetamine-related transcripts	NPY	neuropeptide Y
CORT	corticosterone	NREM	non-rapid eye movement
EEG	electroencephalogram	OSAS	sleep apnea syndrome
EMG	electromyogram	PeN	periventricular nucleus of the hypothalamus
GH	growth hormone	PGO	ponto-geniculo-occipital
GHRH	growth hormone releasing hormone	POMP	proopiomelanocortin
GHS-R1a	growth hormone secretagogue receptor 1a	PPT	pedunculopontine tegmental nucleus
icv	intracerebroventricular	PVN	paraventricular nucleus
ip	intraperitoneal	REM	rapid eye movement
iv	intravenous	SRS	sleep-regulatory substances
LC	locus coeruleus	SST	somatostatin
		SWA	slow-wave activity

Despite its widespread and important physiological actions, ghrelin gene precise transcriptional and translational regulatory mechanisms remain ambiguous. Further studies on the biogenesis, expression and functions of C-ghrelin and obestatin, and the identification of their receptors are required.[8]

Before entering to this reviews' topic, we consider important to describe the phenomenology of the sleep—wake cycle. According to a simple behavioral definition, sleep is a reversible behavioral state of perceptual disengagement and unresponsiveness to the environment.[12] Today it is universally accepted that mammals present at least two basic stages of sleep: non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. The electrographic signals of cortical activity (electroencephalogram (EEG)), eye movements and muscle tone (electromyogram (EMG)) are the major signals, which determine a given sleep stage.[12] In humans, NREM sleep has been further divided into N1, N2 and N3 stages based on specific EEG patterns. The N2 and N3 stages have been characterized by the presence of slow (delta) waves in the EEG and thus, referred also as slow-wave sleep (SWS).[13] However, in the animal literature, the terms SWS and NREM sleep have been used interchangeably and often refer to the same sleep stage (non-REM sleep).[14]

NREM sleep in humans is associated with fragmented mental activity. REM sleep, by contrast, is defined by EEG activation, muscle atonia, and episodic burst of rapid eye movements. The mental activity during REM sleep is associated with dreaming, based on vivid dream recall reported after approximately 80% of arousals from this sleep state.

There is a large body of evidence demonstrating that sleep is influenced by a number of hormones and peptides, referred to as sleep-regulatory substances (SRS). Some of these peptides tend to accumulate within the brain and cerebrospinal fluid. Cerebrospinal or brain extracts taken from a sleep-deprived animal or from animals in the sleep-intense part of the cycle, promote sleep when injected into the ventricles of a normal animal, as demonstrated by several groups.[15–17] Thus, chemicals of different molecular sizes have been suggested to function as neurotransmitters, neuro-modulators or neurohormones, providing the possibility for short-to-long acting molecules that could participate collectively in the generation and maintenance of the sleep—wake cycle.[17–19] Among these SRS, interleukin-1β, tumor necrosis factor α, growth hormone releasing hormone (GHRH), prolactin, and nitric oxide, are currently the best characterized; and many of their downstream biochemical mechanisms are also implicated in sleep regulation, e.g., adenosine, nitric oxide, prostaglandins, and others.[17] However, as discussed below, ghrelin, although it does not meet all the criteria established for SRS,[17,18] is significantly involved in regulating the sleep—wake cycle, in addition to its role in metabolism regulation.

Ghrelin's role on the sleep—wake cycle

Studies conducted in rodents indicate that central administration of ghrelin to rats and mice increases wakefulness, but the effects of systemic ghrelin administration are less clear, and depend on the species, the dose and route of administration (Table 1). On the other hand, the effects of ghrelin administration to humans depend on the gender and time of administration. Repeated intravenous administration of ghrelin increases NREM sleep in young and elderly men, but has no effect on women (Table 2). These studies are discussed in detail in the following pages.

Table 1
Effects of ghrelin administration on the sleep-wake cycle in rodents.

References	Species	Route of administration	Wake	NREM	REM
Tolle et al., 2002[23]	Rat	Repeated iv	Increase	Decrease	Decrease*
Szentirmai et al., 2006[20]	Rat	icv	Increase	Decrease	Decrease
Szentirmai et al., 2007[21]	Rat	Injection into Lateral Hypothalamus	Increase	Decrease	Decrease
Obál et al., 2003[26]	Mice	ip	No effect	Increase	No effect
Szentirmai et al., 2007[30]	G-KO mice		Increase	Decrease	Slight increase
Esposito et al., 2012[32]	GR-KO mice		No effect	No effect	No effect
Szentirmai, 2012[22]	Mice	icv	Increase	Decrease	Decrease
		ip	No effect	No effect	No effect

G-KO= preproghrelin knockout; GR-KO= ghrelin receptor knockout; icv= intracerebroventricular; ip= intraperitoneal; iv= intravenous; NREM= non-rapid eye movement; REM= rapid eye movement.
* Only in the dark period.

Table 2
Effects of ghrelin administration on the sleep-wake cycle in humans.

References	Gender	Route of administration	Wake	NREM	REM
Weikel et al., 2003[35]	Young male	Intravenous	No effect	Increase	Decrease
Kluge et al., 2007[42]	Young female	Intravenous	No effect	No effect	No effect
Kluge et al., 2008[36]	Young male	Intravenous	No effect	Increase	Decrease
Kluge et al., 2010[43]	Elderly male	Intravenous	No effect	Increase	Decrease
Kluge et al., 2010[43]	Elderly female	Intravenous	No effect	No effect	No effect

NREM= non-rapid eye movement; REM= rapid eye movement.

Studies in animals

Most of the advance of ghrelin's role on the sleep–wake cycle in animals has been done by a series of excellent studies conducted in rodents by the group of J.M. Krueger at Washington State University. They have shown that intracerebroventricular (icv) microinjections of 0.2, 1 or 5 μg of ghrelin stimulated wakefulness, suppressed NREM and REM sleep in rats.[20] In addition, microinjections of ghrelin (0.2 or 1 μg) into hypothalamic sites involved in the regulation of sleep–wake cycle or feeding, such as the lateral hypothalamus (LH), medial preoptic area (MPA), and paraventricular nucleus (PVN), promoted wakefulness during the first hour after injection.[21] The 0.2 μg ghrelin microinjection dose increased the time spent in wakefulness when injected into these brain areas, with the exception of the PVN. The dose of 1 μg had similar effects, increasing wakefulness and decreasing both NREM and REM sleep. These effects were accompanied by an increase in food consumption.[21]

Similar results have been obtained in mice, where icv injection of ghrelin increased wakefulness and suppressed NREM sleep and EEG slow-wave activity in the first hour after injections.[22] REM sleep was decreased for 2–4 h after each dose of ghrelin (0.2, 1 or 5 μg). Systemic administration of ghrelin up to 400 μg/kg did not induce changes in sleep–wake activity in mice at dark or light onset. Motor activity and body temperature remained unaltered, and food intake was significantly increased after systemic injections of ghrelin given prior the light period.[22] The author suggest that the activation of central, but not peripheral, ghrelin-sensitive mechanisms elicits arousal in mice, and that circulating ghrelin does not activate the same central wake-promoting mechanisms stimulated by the brain-derived ghrelin, and accessible to centrally injected peptide.[22]

However, repeated intravenous (iv) administration of ghrelin in rats (10 μg) at 3- to 4-h intervals increased wakefulness and decreased NREM sleep during the light period and at the onset of dark period, while REM sleep decreased only during the dark period. The increase of wakefulness and the decrease of NREM sleep after ghrelin administration correlated with an increase in food intake.[23] Moreover, within 30 min after the last ghrelin injection the total time of wakefulness and NREM sleep recovered to basal values whereas REM sleep remained decreased.[23] This method of pulsatile administration, by repeated iv bolus injections mimics the physiological release of the hormone, and appears to be crucial to detect sleep-effects of neuropeptides.[1,2] It is also possible that the wake-promoting effects of ghrelin are due to its stimulatory actions on hypothalamic orexin and neuropeptide Y (NPY) neurons.

The results obtained by centrally or repeated iv administration of ghrelin in rodents suggest that ghrelin is an inhibitor of REM sleep, perhaps by antagonizing somatostatin (SST). Indeed, SST facilitates REM sleep in rats[24] and microinjections of SST antagonist into the locus coeruleus (LC) nucleus decrease REM sleep and prevent REM sleep rebound after 24 h of REM sleep deprivation.[25]

Contrary to the studies just discussed, a single study conducted in rodents has shown that intraperitoneal injection of ghrelin (400 μg/kg) in control mice induced a significant increase in NREM sleep and sleep latency during the first hour.[26] The discrepancies between this result and other studies have been properly discussed by Szentirmai.[22]

On the other hand, ghrelin administered directly to slices of pedunculopontine tegmental nucleus (PPT) and laterodorsal tegmental nucleus (LDT) produces postsynaptic depolarization in dose-dependent manner.[27,28] PPT and LDT nucleus are the main brain stem areas involved in the REM sleep generation.[29] As previously mentioned, central administration of ghrelin decreases REM and NREM sleep in rats and mice.[20–22]

To further explore the role of ghrelin in sleep regulation, ghrelin knockout (KO) mice have been used. Preproghrelin KO mice showed a slight increase in REM sleep and reduced NREM sleep at the expense of increased wakefulness.[30] KO mice had more fragmented NREM sleep than WT mice, with shorter and greater number of NREM sleep episodes.[30]

The authors proposed several explanations to these unexpected findings, considering that ghrelin is involved in promoting arousal, including the lack of obestatin, which induces sleep in rats,[11] in preproghrelin KO mice, redundancy of the arousal system, or the activation of compensatory mechanism during development that allow normal sleep–wake regulation in perproghrelin KO mice.[30] Compensatory mechanisms, such as increase in SST production or increased neuronal activity of PPT or other pontine nucleus, could be responsible for the increase in REM sleep in ghrelin KO mice.

Under thermoneutral conditions (30 °C ± 1) with food *ad libitum* preproghrelin KO mice are able to mount adequate rebound sleep in response to sleep deprivation.[30] However, when preproghrelin KO mice, but not ghrelin receptor KO, were challenged to cold temperatures and fasting, they showed remarkable differences to WT mice. Preproghrelin KO mice showed reduced body temperature and NREM sleep during the first three days under cold temperatures when food was provided *ad libitum*. The decrease in NREM sleep was due to the shortening of the NREM sleep episodes. But under fasting conditions, these KO mice were unable to maintain normal body temperature and lost EEG-identifiable vigilance states.[31] Pretreatment with obestatin, delayed but did not prevent the hypothermic response of preproghrelin KO mice. In addition to ghrelin and obestatin, the mRNA from the ghrelin gene may encode other peptides,[8] any of these could contribute to the deficits observed in preproghrelin KO mice.[31] Ghrelin receptor KO (GR-KO) mice, did not show impaired responses.[31] These results indicate that the presence of functional ghrelin receptors is important for ghrelin to elicit its effects on sleep and thermoregulation in mice.

Although GR-KO mice did not show significant differences in the spontaneous sleep–wake cycle compared to wild type mice, they did not respond after exposure to a novel environment, such as providing a clean cage with fresh bedding, which induces arousal lasting for several hours in WT mice. These results suggest that ghrelin receptive mechanisms play a role in the function of the arousal system.[32]

Results obtained with KO mice should be carefully interpreted. Deletion of a single gene does not necessarily cause major physiological changes, redundant or compensatory mechanisms could take place during development. As discussed by Szentirmai et al.[30] several mice strains with the genetic deletion of an arousal-promoting mechanism, e.g., those lacking orexin, 5HT1B receptors,

histamine H1-receptors, norepinephrine, or histamine, exhibit near-normal duration of sleep. The consequence of single gene disruption on feeding or vigilance behavior is often ambiguous because of developmental compensation or the activation of redundant mechanisms. Interestingly, the normal sleep time is often accompanied by fragmented sleep architecture in some of these and other KO mice.[30]

On the other hand, rats in basal conditions of sleep show the maximum peak of ghrelin secretion 5 h after light onset, whereas the eating peak was 1 h after dark onset.[33] However, when food was available only during the light period, rats ate more during the first hour, while ghrelin's maximum level was observed 3 h before the end of the dark period.[33] These results suggest that ghrelin's peak preceded the food consumption, maintaining a strong dependence with the light cycle in the control group, while food restricted animals did not show this dependence.

In the same experiment food restriction did not change the diurnal rhythm of NREM sleep. However, the total time of NREM sleep during the first hour of the light period decreased by 11%, whereas it increased by 10% during the dark period. REM sleep decreased during the light period and increased during the dark period, without affecting the total time of REM sleep.[33] These results suggest that consummatory behavior is a factor that can modify the diurnal rhythm of both NREM and REM sleep.

Five hours of sleep deprivation carried out during the light period increased ghrelin plasma levels significantly during the first hour of sleep deprivation. Hypothalamic ghrelin content also increased along the duration of sleep deprivation and dropped below baseline after sleep deprivation. In addition, plasma corticosterone (CORT) levels were also increased after sleep deprivation.[33] These results suggest that CORT might stimulate ghrelin's secretion during sleep deprivation. For that reason, sleep-deprived animals increase food intake significantly after the sleep deprivation period. Increased food intake could be a consequence of ghrelin's accumulation.

In addition to the studies just described, it has been shown that the wake-promoting effects of short-term restriction feeding were attenuated in preproghrelin KO animals, but they displayed normal food anticipatory activity, suggesting that ghrelin is not required for the normal function of the food entrained oscillator but it plays a significant role in arousal responses to fasting.[34] Similar to findings in preproghrelin KO mice, orexin neuron-ablated mice show no appreciable increase in arousal during fasting. Despite the profound effects of ghrelin on feeding, preproghrelin and ghrelin receptor KO mice have normal food intake and body weight.[34] Preproghrelin KO and WT mice are able to adapt to scheduled feeding.

Studies in humans

A series of elegant studies conducted in recent years by A. Steiger and colleges in Germany have demonstrated that ghrelin is a sleep-promoting factor in human males (see Table 2). Similarly to GHRH, repetitive intravenous (iv) ghrelin administration enhanced NREM sleep, slow wave-activity (SWA), and plasma GH levels in young normal human males.[35,36] When ghrelin was administered in the early morning, it increased the secretion of cortisol and GH without affecting sleep in 12 healthy male volunteers.[37] However, ghrelin plasma levels increased earlier by a non-significant trend, already before the beginning of sleep recovery after one night of sleep deprivation.[38] When sleep-EEG and nocturnal levels of ghrelin were examined simultaneously, a trend was found suggesting a lower time spent in N1 sleep stage in subjects with high nocturnal ghrelin levels.[39] However, the authors concluded that under normal conditions plasma ghrelin levels show no interaction with sleep patterns.[39] In a different study, ghrelin levels rose sharply around midnight and then slowly declined until the next morning in young non-obese human males.[40] In contrast, when subjects were sleep deprived, ghrelin levels increased steadily up to a plateau in the early morning hours and declined just after breakfast.[40]

The sharp increases of ghrelin before meals may promote hunger and food intake, whereas the nocturnal ghrelin rise observed in studies conducted with human males maybe related to sleep.[39–41] A positive correlation between the nocturnal GH peak and ghrelin levels between 23:00 and 03:00 h has been also found,[40] and suggested by findings from Cummings et al.[41] It appears likely that ghrelin and GHRH are cofactors in sleep regulation.

Furthermore, repeated iv ghrelin administration increases NREM sleep and decreases REM sleep in healthy young men but does not affect sleep in young women.[36,42] Similar gender differences have been reported in elderly men and women.[43] Since the effects of ghrelin on sleep-EEG were absent in premenopausal and postmenopausal women as well, it is unlikely that estrogens contribute to this sexual dimorphism.[2] Ghrelin stimulates the activity of the somatotropic and the hypothalamic–pituitary–adrenal axis regardless of gender. These two endocrine axes are crucially involved in sleep regulation. A major surge of GH secretion is associated with NREM sleep, particularly with deep REM sleep, in various species.[44]

Integration of ghrelin in the sleep–feeding circuit

Taking into account its role in the feeding behavior, the main function of ghrelin in sleep could be related to the maintenance of metabolic homeostasis during this process. Sleep perturbations are frequently associated with alterations in feeding behavior, like hyperphagia and pathologies such as obesity and diabetes.[45] In this sense, ghrelin could interact with a series of molecules regulating the feeding–sleep circuit, among which GH, leptin, and orexins (hypocretins) are of importance. In the following sections we describe these possible interactions of ghrelin in the regulation of the sleep–feeding circuit.

Ghrelin and GH

Although it is obvious that sleep plays an important role in energy balance, the role of sleep in modulating caloric intake is, in fact, very limited. Ghrelin stimulates the release of GH from pituitary cells,[46,47] as well as that of GHRH and SST, which inhibit its secretion. GHRH is mainly expressed in the arcuate nucleus of the hypothalamus (ARC); whereas SST is maximally expressed in the periventricular (PeN) nucleus of the hypothalamus.

The ultradian secretion of GH is linked to the sleep–wake cycle.[48] A major peak of GH secretion occurs during NREM sleep in humans and in some animal species.[44] The role of this burst of GH seems to be crucial in the maintenance of the sleep–wake cycle, since its alteration produces sleep perturbations. Whether these sleep alterations are the result of a direct action of GH is not clear. It has been proposed that GHRH, a SRS, might mediate the NREM sleep-effects of GH.[49] However, the sleep-effects of GH and GHRH appear to be independent since hypophysectomy blocks the GHRH-induced GH release, but has no effect on sleep; while disruption of sleep by environmental stimuli may not interfere with GH secretion (reviewed by Krueger et al.[50]). On the other hand, the rise of GH during sleep is not blocked by the use of SST antagonists, suggesting the participation of an additional GH regulator. As mentioned above, a positive correlation between the nocturnal GH peak and ghrelin levels has been reported.[40] Moreover, Nass and co-workers[51] showed a strong positive correlation between acyl-ghrelin, the active form of ghrelin, and plasmatic levels of GH

during the night period. The relevance of ghrelin and GH association during the first hours of sleep is not clear, although recent evidence suggest that acyl-ghrelin could regulate the secretion of GH in order to maintain the metabolic homeostasis. In this context, Zhao et al.[52] have reported that GH is required to maintain normal blood glucose levels in animals subjected to a caloric restriction regimen. This result is relevant considering that during sleep an extended period of fasting must be maintained overnight. In normal subjects, during overnight sleep, blood levels of glucose remain stable or fall only minimally despite the extended fast.[53] Thus, a number of mechanisms operating during nocturnal sleep must intervene to maintain stable glucose levels regardless of overnight fast. The maintenance of glucose level during normal sleep could be related to the peak of GH found in the first half of sleep, since this hormone is able to exert direct metabolic effects on fat and muscle tissues. It has been suggested that GH acts as a counter-regulatory hormone, antagonizing the hepatic and peripheral effects of insulin on glucose metabolism, through mechanisms involving the concomitant increase in free fatty acids release and uptake.[54] This ability of GH to induce insulin resistance is significant for the defense against hypoglycemia during sleep. Taken together, these facts might explain the elevated levels of ghrelin (an orexigenic hormone) observed during early sleep. It is plausible that ghrelin modulates GH nocturnal secretion to regulate glucose metabolism during sleep (see Fig. 1).

Ghrelin and leptin

Leptin is a protein of 167 amino acids, produced mainly by the adipose tissue.[55] Initial studies, investigating the physiologic role of leptin in mice, demonstrated that this protein was directly involved in the regulation of satiety, energy and feeding behavior.[56] The ob/ob mice, which do not produce functional leptin, become obese when they are fed ad libitum.[57] Administration of leptin reversed this weight gain.[57] However this encouraging result did not extrapolate to obese humans, because leptin resistance is observed in these subjects.[58] There is only one successful case of weight loss after recombinant leptin replacement therapy in an obese child with congenital leptin deficiency.[59] It has been reported that circulating leptin concentrations in humans show a rapid decline or increase in response to acute caloric shortage or surplus, respectively.[60] These changes in leptin levels have been associated with reciprocal changes in hunger due probably to an increase in circulating ghrelin.

In a similar way to ghrelin, leptin might participate in the regulation of sleep by decreasing REM sleep and influencing NREM sleep.[61] The 24-h leptin profile shows a marked nocturnal rise, which is partly dependent on meal intake.[62] Also, it was reported that levels of leptin are higher during sleep than in wakefulness.[63] Plasma levels of leptin might be influenced by the energy balance of the organism and by sleep length as described for ghrelin. Chronic and partial sleep deprivation, as well as acute sleep deprivation, might cause a reduction of leptin concentration in serum.[64] As mentioned above, glucose levels during sleep are homeostatically controlled; however, sleep loss has an adverse impact on glucose regulation.

The first attempts to examine the glucose tolerance (capacity of the cell to metabolize glucose) or food intake during sleep deprivation were based on the total sleep deprivation paradigm for extended period of time.[65] Despite the importance of the results obtained with this approach, these observations did not receive sufficient attention, probably because such total sleep deprivation
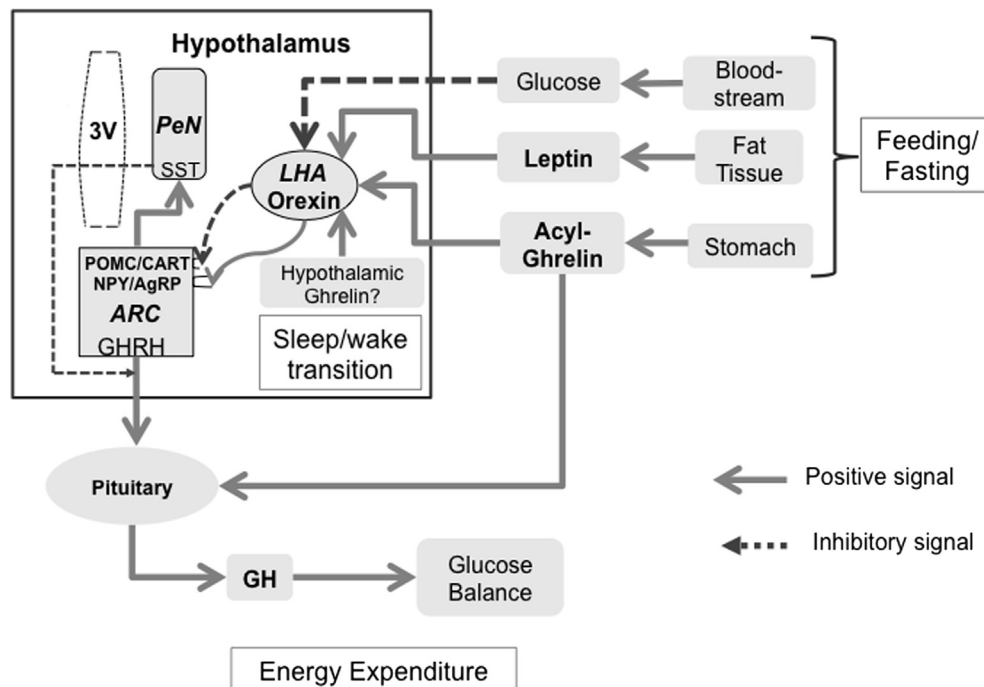

Fig. 1. Possible ghrelin interactions in the feeding and sleep circuits. Feeding or fasting produce changes in glucose, leptin, and ghrelin levels, which could affect the sleep to wake transition. Acyl-ghrelin appears to regulate nocturnal GH secretion through a direct action on the pituitary gland. This GH increase could be essential for glucose homeostasis during sleep. Gastric- or perhaps hypothalamic-produced ghrelin also activates orexin neurons in the LHA, which in turn activate NPY/AgRP neurons in the ARC (ghrelin orexigenic pathway). At the same time, this orexigenic stimulus produces secretion of SST that inhibits GHRH action, blocking the production of GH in the pituitary. The net effect is a promotion of arousal and the consummatory behavior. On the other hand, sleep perturbations induce elevation of ghrelin with a decrease of leptin levels that directly increase the activity of the orexin system, affecting the sleep–wake transition status of animals and their consummatory behavior. 3V: 3rd ventricle. ARC: arcuate nucleus of the hypothalamus. GH: growth hormone. GHRH: GH releasing hormone. LHA: lateral hypothalamic area. NPY/AgRP: neuropeptide Y/agouti-related peptide. PeN: periventricular nucleus of the hypothalamus. POMC/CART: proopiomelanocortin/cocaine and amphetamine-related transcripts. SST: Somatostatin.

is very unlikely to occur in real life. In this way, the decrease of sleep hours rather than total deprivation (sleep restriction paradigm), better reflects sleep disturbances in humans and it has been used to understand the metabolic regulation during sleep. Sleep restriction produces a decrease of glucose tolerance, causing an elevation of glycemia. The profiles of ghrelin and leptin, hormones that regulate feeding behavior, are modified during sleep restriction. Several studies have shown that both, chronic and partial sleep deprivation,[66] and acute sleep deprivation[67] might cause a reduction in the serum concentration of leptin. Compared to normal sleep (8 h), a 4 h sleep restriction for six nights lowers leptin levels by 19 and 26% for mean and maximum values, respectively.[66] These results suggest that sleep plays an important role in leptin secretion. Sleep restriction seems to change the capacity of leptin to respond to the body's energy balance and to produce the satiety signal when energy needs have been adequately met.[66]

Similar to the case of leptin, sleep seems to influence the pattern of ghrelin secretion, since high levels of this hormone are observed during sleep curtailment in human subjects.[66] Sleep curtailment of 4 h in twelve healthy men for a period of two days was associated with an increase of almost 28% in the diurnal levels of ghrelin.[64] On the other hand, a single night of sleep deprivation increases ghrelin and feeling of hunger in healthy men.[68] A similar increase in plasma and hypothalamic concentrations of ghrelin was documented in rats after sleep restriction.[33] Taken together these results suggest that high levels of ghrelin and low levels of leptin, occurring in response to sleep restriction, might be a normal response of the body to a greater need for energy intake. Therefore, high ghrelin levels can contribute to an increase of hunger and food intake, while reduction of leptin increases energy needs, due to the increase in wake time (Fig. 1).

Possibly because of their alteration during sleep restriction, ghrelin and leptin are related to the obstructive sleep apnea syndrome (OSAS). This syndrome is characterized by repetitive collapse of the upper airway during sleep, producing numerous episodes of arousal. OSAS is associated with obesity, which is the main risk factor to develop this syndrome. Several studies have shown an alteration in the levels of ghrelin and leptin associated with OSAS; however, it is not clear whether this modification is related to the role of these hormones in the body's metabolism or due to the OSAS syndrome itself.[69–71] Altogether, these results strongly suggest a delicate balance between anorexic and orexigenic signals to maintain the metabolic status during sleep.

Ghrelin and orexins (hypocretins)

From the evidence described above, it is clear that there is a neuronal circuit regulating both sleep and metabolism, where ghrelin plays an important role. The hypothalamus is an important brain area that regulates several homeostatic processes, including energy balance. Neurons in the arcuate nucleus of the hypothalamus act as sensors of circulating hormones. A group of arcuate neurons co-expresses NPY and agouti-related peptide (AgRP), while another group co-expresses proopiomelanocortin (POMC) and cocaine and amphetamine-related transcripts (CART). In this circuit, ghrelin stimulates the NPY/AgRP neurons sending an orexigenic signal, while leptin inhibits them and activates the POMC/CART neurons producing an anorexigenic response.[72,73] Thus, activation of NPY/AgRP and POMC/CART neurons has orexigenic and anorexigenic properties, respectively.

How is this neural circuit regulated? Orexins, also called hypocretins, are neuroexcitatory peptides produced in the lateral hypothalamic area.[72] Two types of orexin receptors, orexin-A and orexin-B, have widespread distribution through the brain including the hypothalamus. Deficiency in the orexin system has been linked

to narcolepsy in humans, dogs and mice, a chronic neurological disorder resulting in excessive daytime sleepiness, cataplexy and fragmented sleep. These peptides have been related to the regulation of the sleep–wake cycle, arousal and energy homeostasis. The orexin system also acts as an arousal sensor through its sensitivity to circulating metabolic factors (leptin, ghrelin, glucose), since their concentration correlate with modification of arousal (reviewed elsewhere[72,73]). Decreased leptin levels and increased glucose and ghrelin levels directly modulate orexin activity, by increasing orexin neurons excitability. Thus, enhanced orexin neuronal activity may promote consummatory behaviors, arousal and increased energy expenditure by activation of NPY/AgRP neurons (ghrelin orexigenic pathway) and indirect inhibition of POMC/CART cells (leptin–anorexigenic pathway).[74]

The orexin system may provide intra and extrahypothalamic target circuits with local sensor circuits that detect subtle changes in metabolism at arousal. Interestingly, orexin neuron projections are found in the PeN and ARC, two "classical" nuclei controlling GH secretion from the anterior pituitary. The participation of orexin in GH secretion is not yet clear; some authors suggest a role of orexin in GH release via GHRH in ovine somatotroph cells in culture, while other studies were unable to show any such effect of orexin in vivo, although it markedly blunts GH response to ghrelin (reviewed in [75]). Although the physiological significance of the orexin-GH axis interaction is still uncertain, it is possible that orexins could act as a signal linking metabolic and nutritional status, as well as hormones (ghrelin and leptin) and nutrients such as glucose, with the somatotropic axis. A possible integration among these sleep and feeding signals is summarized in Fig. 1. It has been suggested that reduction of sleep time as a result of modern life is accompanied by alteration in the balance of ghrelin, leptin, GH, and glucose levels, which might be related with the development of obesity and diabetes.[76]

Sleep restriction, leptin–ghrelin balance and obesity

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems.[77] Obesity is a rapidly spreading epidemic in the majority of developed countries.[78] Obesity adversely affects health by increasing the risk for various associated conditions including the metabolic syndrome, type 2 diabetes, coronary artery disease, and hypertension; all of them are associated with increased mortality.[79]

It has been reported an association between sleep restriction and obesity in humans.[80,81] During the past few decades, sleep curtailment has become a very common behavior in contemporary societies. Survey studies conducted in the United States found modal sleep duration of 8–8.9 h in 1960,[82] 7 h in 1995,[83] and 6 h or less in 2004.[84] In recent years more than 30% of adult men and women in the USA (30–64 y old) report sleeping fewer than 6 h per night.[84] Coincidently, this trend for shorter sleep duration has developed over the same period of time as the dramatic increase in the prevalence of obesity and diabetes. Evidence is rapidly accumulating to indicate that chronic and partial sleep loss may increase the risk of obesity and diabetes.[85]

Cross-sectional epidemiologic studies conducted in adults from different countries have all observed a significant association between short sleep duration and obesity, both in adults and children (reviewed in[85]). Some studies have shown a U-shaped relationship between sleep duration and body mass index (BMI), the minimum BMI was observed in those individuals reporting 7–8 h of sleep per night.[86] Longitudinal studies in the United States have also indicated that adults sleeping less than 6 or 7 h per night were at risk of gaining weight and had higher BMI.[87–89]

The two key opposing hormones in appetite regulation discussed above, leptin and ghrelin, play a significant role in the interaction between short sleep duration and high BMI. As mentioned, leptin is an adipocyte-derived hormone that suppresses appetite,[56] while ghrelin is predominantly a stomach-derived peptide that stimulates appetite.[3] Short sleep duration has been associated with decreased leptin and increased ghrelin levels and hunger,[64,80] changes that have also been observed in reaction to food restriction and weight loss, and are typically associated with increased appetite.[81] Alterations on these hormones may contribute to the increase in BMI that occurs with sleep curtailment. The changes in leptin and ghrelin with sleep restriction could, therefore, provide a powerful dual stimulus for food intake that may culminate in obesity.

An up-regulation of the activity of orexin neurons can be a primary mechanism linking sleep deprivation and adverse metabolic effects. Decreased circulating leptin levels and increased glucose and ghrelin levels directly modulate orexin activity by increasing neuronal excitability.[72,73] Thus, enhanced orexin neuronal activity could promote feeding behavior. The orexin system acts as a sensor of internal state (metabolism and arousal) in promoting arousal.[72,73]

Less time of sleep also allows for more opportunity to eat. Sleep restriction or habitual short sleep duration increases hunger/ appetite, and increases food intake and snack consumption.[80] Along these lines, studies performed with rodents showed that rats fed a high protein-to-calorie diet had accelerated weight loss, as compared to sleep-deprived rats fed calorie-augmented (fatty) diets.[90] Food consumption remained normal in sleep-deprived rats fed protein-rich diets, but increased 250% in rats fed calorie-rich diets. Preference for fatty foods has also been reported in sleep-deprived humans.[90] Sleep deprivation may thus increase not only appetite but also preference for lipid-rich, high-calorie foods. In societies where high-calorie food is freely available and consumption uncontrolled, after milder chronic sleep restriction, the equation may be tipped toward food intake for high-calorie food rather than expenditure, culminating in obesity. Short sleepers may also have more time to overeat.[80,83]

Thus, through these pathways, sleep loss could lead to increased appetite and increased food intake, which could lead to obesity. Finally, sleep loss and the associated sleepiness and fatigue may result in reduced energy expenditure, in particular through decreased physical exercise, but also through decreases in non-exercise activity thermogenesis. Reduced energy expenditure is to date an unexplored pathway that could link short sleep and the risk of overweight and obesity.[85]

Conclusions

The findings discussed in this review suggest that the physiological effects of ghrelin depend on the route and time of administration, the dose injected, the species, and gender. In rodents, central administration of ghrelin induces wakefulness, whereas the effects of systemic administration are less clear. A single dose of intraperitoneal (ip) ghrelin administration had no effect in rodents, while repeated iv injections induced wakefulness in rats. In human males, on the contrary, repeated iv ghrelin injections promote sleep, but have no effect on women. Similar to GH, there is an increase of ghrelin levels during sleep. Since a number of mechanisms operating during nocturnal sleep must intervene to maintain stable glucose levels, regardless of overnight fast, e.g., GH release, it is possible that ghrelin modulates GH nocturnal secretion to regulate glucose metabolism during sleep. The studies reviewed here also suggest that sleep curtailment, a trend in contemporary societies, decreases leptin and increases ghrelin plasma levels, which in turn up-regulate appetite, representing a risk for obesity and a reduction of insulin sensitivity.

Research agenda

1) To determine the effects of ghrelin administration into the brain on the synthesis of different neurotransmitters.
2) To determine the role of ghrelin on the metabolic status of organisms during sleep.
3) More studies to understand the interaction between ghrelin, GH, leptin, and orexins in the metabolic equilibrium during sleep.
4) To investigate the role of ghrelin's rise and leptin's decrease in sleep restricted subjects and its role in obesity.
5) High levels of ghrelin and low levels of leptin occurring in response to sleep restriction might be a normal response of the body to a greater need for energy intake. Therefore, high ghrelin levels can contribute to an increase of hunger and food intake, while reduction of leptin increases energy needs, due to the increase in wake time. Then it will be important to measure ghrelin and leptin plasma levels in patients with sleep disorders, as well as in sleep-restricted humans.
6) To perform studies in obese humans, especially those with sleep disorders, with protein-rich diets *vs* fat-rich ones, in order to show if they promote weight loss as demonstrated in rodents.
7) Further studies to understand the transcriptional and translational regulatory mechanisms of the ghrelin gene.

Practice points

1) The effect of ghrelin on the sleep–wake cycle depends on the route of administration, the dose injected, the light cycle, the species, and gender.
2) The gastric hormone ghrelin stimulates the release of the GH from pituitary cells, and induces an increase in the metabolism.
3) Together with GH, leptin, and orexins, ghrelin might regulate the metabolic status of the organisms during sleep.
4) Several studies have indicated that adults sleeping less than 6 or 7 h per night are at risk of gaining weight and increase their BMI. Shorter hours of sleep can promote more opportunity to eat and gain weight.
5) Recommend patients to avoid late night leisure activities that promote sleep curtailment.

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