Effects of nutrition on neuro-endocrine stress responses
Nicolas Rohleder\textsuperscript{a,b} and Clemens Kirschbaum\textsuperscript{a}

Purpose of review
Past studies in humans and animals have shown that low blood glucose concentrations due to fasting negatively interfere with the ability to mount a hypothalamus–pituitary–adrenal (HPA) axis response to psychological stress or to pharmacological activation, respectively. This contradicts the classical view of the proposed primary glucocorticoid function of providing the individual with energy in fight-or-flight situations.

Recent findings
Not many studies have followed up on this phenomenon in recent years, but our understanding of how appetite and satiety is regulated has significantly improved. Many of the neuropeptides involved in regulation of energy homeostasis interact with key areas of the HPA axis. The majority of orexigenic peptides have been shown to activate the HPA axis, while some anorexigenic peptides negatively modulate HPA axis activation and others also stimulate it.

Summary
The effects of orexigenic peptides on the HPA axis are incompatible with the phenomenon of blunted HPA axis activity in states of low energy available, while the fact that some anorexigenic peptides activate the HPA axis might point to a permissive role. In conclusion, current data insufficiently explain negative HPA axis modulation by low glucose levels.

Keywords
anorexigenic, HPA axis, neuropeptides, orexigenic, stress

Abbreviations
ACTH adrenocorticotropic hormone
AgRP agouti-related protein
ARC arcuate nucleus
CART cocaine and amphetamine related transcript
CRH corticotropin-releasing hormone
GLP-1 glucagon-like peptide-1
HPA hypothalamus–pituitary–adrenal
LHA lateral hypothalamic area
MCH melanin concentrating hormone
MSH melanocyte stimulating hormone
NPY neuropeptide Y
POMC pro-opiomelanocortin
PVN paraventricular nucleus

Introduction
Glucocorticoids, the main effectors of the hypothalamus–pituitary–adrenal (HPA) axis, received their name for their ability to stimulate gluconeogenesis in the liver (‘gluco-’) and for being synthesized in the cortex of the adrenal glands (‘-corticoids’) [1]. Although we know today that glucocorticoids exert a plethora of effects throughout the organism [2], the general view remains that glucocorticoids are required to release glucose during situations of stress, to enable us to be ready for fight-or-flight responses.

Most interestingly, this latter function has been questioned by results of human and animal studies conducted in the past 15 years. Based on their theory that circadian rhythms of feeding are associated with circadian rhythm of the HPA axis [3], Hanson et al. [4] studied HPA axis responses of rats to 30 min of physical restraint either after a 17-h fast during the activity period (during the night) or fed \textit{ad libitum}. Fasted rats were not able to activate their HPA axis, as shown by unchanged concentrations of plasma adrenocorticotropic hormone (ACTH). Further experiments using a 14-h fasting period showed that this blunting of HPA axis activity was more pronounced when fasting occurred during the nightly activity period, as compared to fasting during the day (i.e. the inactive period). Supply of calories partly restored the ACTH response. In another set of studies published by the same group [5], the nonresponse of plasma ACTH in fasted rats was confirmed, although corticosterone responses did not differ between fed and fasted animals. It was further shown that fasting itself activated the HPA axis.
Two studies from our laboratory later showed that human HPA axis responsiveness is similarly regulated by energy availability. In humans, psychosocial stress can be reliably induced by exposing individuals to a socially-evaluative situation, which is best achieved by a public speaking paradigm such as a simulated job interview [6]. We subjected healthy young men to the Trier Social Stress Test, which is one of the most potent socially-evaluative laboratory stress tasks [7]. Prior to the stress task in the late afternoon, all subjects fasted for 8 h. One group received a standardized glucose drink before stress, while the other group received only water. In line with the animal findings, fasted subjects were not able to activate the HPA axis in response to stress, as evident by blunted free cortisol responses. Subjects in which blood glucose levels had been restored by glucose drink showed normal cortisol increases, and cortisol and blood glucose increases were highly correlated. In contrast to the animal data, our subjects did not show signs of HPA axis activity before the stress task. In an attempt to see whether this response pattern in fed versus fasted states was a general feature of HPA regulation, we applied nicotine as a pharmacological stimulus of the HPA axis. Habitual smokers were asked to smoke two cigarettes in short succession after an 8-h fast. Again, cortisol increases were only observed in those smokers who had received a glucose drink to restore blood glucose levels [8]. Based on these findings, we set out to investigate whether the effect of nutritional restoration on HPA axis responsiveness was restricted to glucose, or could also be achieved with other types of foods. We confirmed our previous findings of a blunted cortisol response after an 8-h fast and showed that only the glucose drink, not a standardized high fat or high protein meal, successfully restored HPA axis responsiveness [9].

These findings clearly contradict the classical view that a main function of stress-induced HPA axis activation and glucocorticoid increase is to provide energy by stimulating gluconeogenesis. Instead it seems that the HPA axis can hardly be activated when blood glucose concentrations are in the low euglycemic range. While these results appear to be consistent in animal and human experiments, the underlying mechanisms are poorly understood. One explanation might be that fasting itself activates the HPA axis, leading to a decreased responsiveness, possibly mediated by negative feedback mechanisms. This is, however, not a very likely explanation because human subjects did not present with an activated HPA axis after an 8-h fast, and still showed blunted responsiveness of the system. Another explanation might be found in the central nervous system, where centers that regulate energy homeostasis are located in close proximity to centers that regulate HPA axis activity, and several neuropeptides involved in regulating energy homeostasis have been documented to influence HPA axis activity.

Modulation of the HPA axis by neuropeptides involved in energy homeostasis and appetite regulation

Since our knowledge on central nervous system regulation of appetite has significantly improved, we will review available data on how peptides involved in appetite and energy regulation modulate HPA axis activity and reactivity. In order to explain the findings of blunted HPA axis responses in states of low energy availability, neuropeptides that stimulate feeding behavior should downregulate, and those that inhibit feeding behavior should upregulate or permit HPA axis activation.

Neuropeptide regulation of appetite and energy homeostasis

Energy homeostasis is regulated in the hypothalamus by a complex network of orexigenic and anorexigenic neuropeptides (see Arora and Anubhuti [10**] for an excellent summary). Several hypothalamic nuclei are involved in appetite regulation. The most prominent is the arcuate nucleus (ARC), which encloses the third ventricle. Due to a less tight blood-brain barrier, the ARC can receive more chemical signals from the periphery, which enables it to act as a feeding control center. The ventromedial nucleus of the hypothalamus (VMH) has been named the satiety center because it appears to be a target for leptin, and lesions to the VMH cause hyperphagia [11]. The lateral hypothalamic area (LHA) has been regarded as a feeding center because it contains glucose-sensitive neurons that are stimulated by hypoglycemia, with lesions suppressing food intake [12]. The dorsomedial hypothalamic nucleus (DMH) also contains leptin receptors and lesions change feeding behavior [13]. The paraventricular nucleus (PVN) is central to HPA axis activation, as it contains the cells secreting corticotropin-releasing hormone (CRH) into the median eminence. The PVN is also implicated in the regulation of energy balance, through receiving projections from the ARC and other energy centers. Apart from the hypothalamus, the nucleus of the solitary tract (NTS) in the brain stem appears to be important in appetite regulation [10**].

Several neuropeptides are involved in regulation of energy homeostasis and some of them were discovered only recently. Central orexigenic peptides are neuropeptide Y (NPY), melanin concentrating hormone (MCH), orexins, agouti-related peptide (AGRP), and galanin. An important peripheral orexigenic signal is ghrelin. Central anorexigenic peptides involve cocaine and amphetamine related transcript (CART), melanocortins (pro-opiomelanocortin; POMC). One of the most important peripheral anorexigenic peptides is leptin, while glucagon like peptide-1 (GLP-1) is produced centrally and in the periphery [10**].
**HPA axis modulation by orexigenic peptides**

Do orexigenic peptides directly or indirectly affect HPA axis activity or reactivity?

**Neuropeptide Y**

Neuropeptide Y is a very potent orexigenic factor that is primarily expressed in neurons of the arcuate nucleus, which project to other feeding centers such as the DMH and LHA, but also to the PVN [14]. NPY containing neurons in the ARC are activated by fasting and stimulate feeding behavior by NPY signaling into the PVN [15]. Intracerebroventricular injection of NPY strongly induces feeding behavior [16]. Since fasting activates NPY signaling to the PVN, it might very well modulate HPA axis responsiveness. Indeed, ARC NPY containing neurons have been reported to project to CRH containing neurons of the PVN [17,18].

A considerable number of studies have been conducted in which laboratory rats were injected with NPY intracerebroventricularly as well as intravenously. The majority of intracerebroventricular studies report that NPY stimulates hypothalamic CRH mRNA or immunoreactivity [19,20], and increases peripheral concentrations of ACTH and corticosterone [21,22,23**]. One study [21] showed that injection of low concentrations of NPY decreased ACTH and corticosterone. In agreement with this latter finding, intravenous application of NPY reduced ACTH and cortisol secretion during nocturnal sleep in humans [24].

**Melanin concentrating hormone**

Melanin-concentrating hormone (MCH) is a recently discovered neuropeptide that appears to be involved in the regulation of feeding. MCH is predominantly present in the LHA and intracerebroventricular injection of MCH stimulates feeding in animals [25]. While peripheral administration of MCH did not impact HPA axis function in rats, it stimulated ACTH release in animals with increased permeability of the blood–brain barrier when injected intracerebroventricularly [26]. In contrast to that, Bluet-Pajot [27] reported that MCH attenuated stress-induced increases of ACTH. In a recent study, Kennedy et al. [28] showed that microinjection of MCH into the PVN increased circulating ACTH and corticosterone concentrations in rats, and co-incubation of hypothalamic explants with MCH stimulated CRH production.

**Orexins**

The orexins (orexin-A and B) are a relatively young group of hypothalamic neuropeptides that were discovered in 1998. Orexins are mainly located in the LHA and orexin-positive neurons show wide projections to intrahypothalamic and extrahypothalamic sites. Orexins are upregulated during fasting and stimulate food intake [29]. Intracerebroventricular injection of orexin-A and B was shown to dose-dependently activate the HPA axis in rats by stimulating CRH release in the PVN. No effects of peripheral application or isolated stimulation of pituitary and adrenal glands could be observed. Orexin-A was a stronger HPA axis activator than orexin-B [30,31]. The HPA axis activating properties of orexin-A were also confirmed in female rats [32]. Since orexin-effects resemble those of NPY, it has been suggested that orexin-effects are mediated by NPY. Orexin-containing neurons project to NPY neurons in the ARC [33], and pretreatment with an NPY receptor antagonist dose-dependently inhibited orexin-A induced HPA axis activation [34].

**Agouti-related protein**

Discovered in 1997, agouti-related protein (AgRP) is another orexigenic neuropeptide [35], which functions as endogenous melanocortin-receptor antagonist [36]. AgRP is coexpressed with NPY in the arcuate nucleus and its expression is increased by fasting [15], and inhibited by leptin [37], respectively. Overexpression of AgRP, which can be the result of leptin-deficiency, causes obesity [37]. AgRP exerts its appetite-stimulating effects mainly by blocking the anorexigenic melanocortin signaling pathway [15].

Since it has been shown that AgRP-positive neurons located in the ARC project to the PVN [15], effects of AgRP on the HPA axis seem plausible; the effects of intracerebroventricular AgRP injections into female rhesus monkeys were tested in two independent studies. Xiao et al. [38] showed that AgRP injections in concentrations similar to those that stimulate feeding induced significant increases of ACTH and cortisol. Parallel infusion of α-melanocyte stimulating hormone (MSH) abolished the AgRP effect. Furthermore, AgRP infusion strongly enhanced the ACTH response to IL-1β, but had no impact on cortisol levels [38]. These results were corroborated by Vulliemoz et al. [39], who showed that five hourly intracerebroventricular infusions increased peripheral cortisol concentrations. Effects were slightly weaker, probably because each of the five AgRP concentrations used was at the lower effective threshold as determined by Xiao et al.

In addition to its effects in the hypothalamus, AgRP exerts a local paracrine/autocrine control on adrenal steroidogenesis [40†]. Long-term treatment (24 h) of bovine adrenal cells with AgRP suppressed ACTH (and α-MSH) stimulated cortisol release; only in low concentrations with no effect in high concentrations [41]. Shorter treatment did not affect ACTH-stimulated cortisol release in an earlier study [42]. In agreement with these findings, Dhillo et al. [43] reported that AgRP administered alone did not affect corticosterone release, but that
Galanin
Galanin is a neuropeptide that was first discovered in the porcine intestinal tract [44], and found to be present in the rat central nervous system, including the PVN, ARC and other hypothalamic nuclei [45]. Microinjections into the PVN showed that galanin stimulates feeding [46], and it appears to preferentially stimulate consumption of high fat foods [47].

While an early study showed that intracerebroventricular galanin injection does not change corticosterone concentrations in male rats [48], Tempel and Leibowitz [49] reported that microinjection of galanin into the PVN decreased peripheral corticosterone concentrations in male rats, but only during the light period. A recent study [50] again reported no effect of galanin microinjections into the PVN on corticosteroid levels, but subcutaneous injections of galanin stimulated ACTH and corticosterone release and potentiated both hormones' increase in response to ether or cold stress [51]. Slightly different results were obtained when tissues of the HPA axis were treated with galanin in cultures. Bergonzelli et al. [52] demonstrated that galanin stimulates CRH and NPY release by rat fetal hypothalamic neurons in culture, and cultured cells taken from the rat adrenal cortex responded with increased corticosterone secretion after co-incubation with galanin [53,54*].

In humans, intravenous galanin infusion did not change corticosterone concentrations of ACTH and cortisol in women [55]. In men, intravenous galanin infusion did not change ACTH and cortisol concentrations either, but if galanin was co-infused with CRH, it diminished CRH-induced increases in ACTH and cortisol [56].

Ghrelin
The peptide ghrelin was discovered in 1999 in the rat stomach and named after its ability to induce pituitary growth hormone secretion [57]. Ghrelin appears to signal appetite to the central nervous system by stimulating the activity of NPY and AgRP containing neurons in the ARC of the hypothalamus [58]. Peripheral and intracerebroventricular application of ghrelin induces weight gain in rodents by reducing fat utilization and by increasing food intake [59]. In the original publication by Kojima et al. [57], ghrelin did not stimulate ACTH release from cultured rat pituitary cells or after intravenous injection into male rats. In the follow-up, however, intravenous injection of ghrelin into healthy human volunteers induced marked increases in ACTH and cortisol in three independent studies [60–62].

Hypothalamic Pituitary Adrenal (HPA) axis modulation by anorexigenic peptides
Do anorexigenic peptides directly or indirectly affect HPA axis activity or reactivity?

Cocaine and amphetamine related transcript
CART is a neuropeptide that was discovered in 1995 [63,64], and functions as a satiety signal. CART gene expression in the hypothalamic ARC decreases during fasting, and intracerebroventricular injection of CART reduces feeding behavior and blocks NPY-induced feeding. CART gene expression in the ARC nucleus is activated by leptin, suggesting that CART links peripheral satiety signaling by leptin to hypothalamic feeding centers by blocking NPY [65].

Koylu et al. [66**] recently summarized the bidirectional interactions of CART and the HPA axis. CART peptides are expressed at every level of the HPA axis: in the PVN of the hypothalamus, the pituitary, and in the adrenal gland. Several studies consistently show that CART has stimulatory effects on the HPA axis. Intracerebroventricular injection of CART into rats was shown to increase CRH, ACTH, and corticosterone [67–71]. The same effect was found in hypothalamic explants, where CART induced CRH expression [68].

Melanocortins
Melanocortins are another group of peptides important in regulation of energy metabolism that are derived from POMC. POMC is a precursor protein that is differentially expressed in neurons to produce a variety of proteins, including α-MSH, β-endorphin, and ACTH. In addition to the pituitary, POMC is expressed in several hypothalamic regions [72]. As Mountjoy and Wong elaborate, melanocortin regulation of appetite is complex and consists of a network of several peptides and receptors [72].

Data on melanocortin effects on the HPA axis are rather inconsistent. Calogero et al. [73] reported that in cultured hypothalamic tissues of the rat, α-MSH decreased stimulated and baseline CRH release. In a more recent study by Dhillon et al. [74], however, stimulation of hypothalamic explants with α-MSH significantly increased CRH release, and microinjections of α-MSH into the PVN of the hypothalamus stimulated increases of peripheral ACTH and corticosterone in rats [74]. Alpha-MSH has consistently been shown to modulate the HPA axis responses to peripheral inflammation. Intracerebroventricular injection into brains of rhesus monkeys and rats prevents IL-1β, IL-6, or endotoxin induced HPA axis activation [75,76**,77,78].

Glucagon-like peptide-1
GLP-1 is derived from the same precursor as the pancreatic glucagon, i.e. preproglucagon. In the small intestine and the central nervous system, however, differential splicing
produces GLP-1 [79,80]. Given intracerebroventricularly, GLP-1 is a strong inhibitor of feeding and drinking behavior [81]. GLP-1 positive cells appear to be located in the NTS, but project to a wide variety of brain areas, one of which is the PVN of the hypothalamus, thus permitting GLP-1 effects on the HPA axis [82]. Larsen et al. [80] reported that intracerebroventricular application of GLP-1 significantly increased plasma corticosterone concentrations in rats. Similar results were reported using a GLP-1 receptor agonist in rats, which stimulated ACTH and corticosterone release [83].

Leptin
Leptin is a peripherally secreted anorexic peptide discovered in 1994 [84]. Leptin is synthesized mainly by adipose tissue and appears to inform hypothalamic centers about the energy stored in the body. Genetic mutations of the ob gene, which encodes for the leptin protein, lead to hyperphagia and severe obesity in animals [85] and humans [86], that can be prevented by leptin administration [86,87]. Leptin concentrations decrease during fasting, and increase after feeding [88]. Leptin effects are mediated in the hypothalamus via downregulation of the orexigenic peptides NPY, MCH, orexins, and AgRP and by upregulation of the anorexigenic peptides α-MSH, CART, and CRH [89].

It appears, however, that leptin rather inhibits HPA axis activity. Leptin downregulates CRH gene expression in the PVN of adrenalectomized mice [90]. While short-term intravenous leptin treatment did not change adrenal cortisol secretion in response to ACTH of rhesus monkeys [91], it did so in human and rat models. In cultured human adrenal cells, leptin pretreatment for 6–24 h prevented an ACTH induced cortisol increase, and it similarly suppressed the corticosterone response in cultured rat adrenal cells [92]. Leptin inhibited ACTH and corticosterone responses to restraint stress in mice, and suppressed the CRH response to lowering of glucose concentrations in cultured rat hypothalamic cells [93]. Leptin intravenously was also shown to inhibit the HPA axis response to endotoxin in female rhesus monkeys [94].

In contrast to these findings, Jang et al. [95] reported that intracerebroventricular leptin infusion stimulates CRH release in adrenalectomized, but not in intact mice. These results have recently been replicated by Jethwa et al. [96] in rats that additionally documented increases of ACTH and corticosterone after intracerebroventricular application of leptin. In summary, results are inconsistent and document leptin-induced activation as well as suppression of the HPA axis.

Conclusion
The orexigenic peptides NPY, MCH, orexins, AgRP, and ghrelin stimulate the HPA axis rather then suppressing it.

Unfortunately, these findings do not readily explain why low blood glucose levels suppress HPA axis activation to psychological and pharmacological stimuli. The only exception might be the inhibition of CRH-induced activation by intravenous infusion of galanin into healthy men. These data, however, stem from only one study and await replication. The anorexigenic peptides CART and GLP-1 seem to exert HPA axis activating properties, which is better compatible with the findings of blunted HPA axis responses in states of low energy availability. It might be speculated that low expression of CART and GLP-1, resulting from low energy availability, would reduce the excitability of CRH-expressing neurons in the PVN. Melanocortins have been consistently shown to attenuate activation of the HPA axis by inflammatory stimuli, which, again, does not help explaining why a low euglycemic state would lead to HPA axis suppression. In conclusion, most of the central and peripheral neuropeptides involved in regulation of energy homeostasis and appetite have some impact on the HPA axis. Unfortunately, very little of these documented effects are compatible with the observation of HPA axis suppression in states of low energy availability. Future studies will therefore have to investigate the modulation of HPA axis activation by energy availability and delineate the impact of specific neuropeptides using methods such as receptor blockade or knockout models.

Acknowledgements
Nicolas Rohleder is supported by grants from the German Research Association (DFG; Ro 2353/4-1) and from the Michael Smith Foundation for Health Research (MSFHR).

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 561).


5 Akana SF, Strack AM, Hanson ES, Dallman MF. Regulation of activity in the hypothalamic-pituitary-adrenal axis is integral to a larger hypothalamic system that determines caloric flow. Endocrinology 1994; 135:1125–1134.


This is an excellent summary of the neuropeptides involved in appetite regulation.


Mizuno TM, Mobbis CV. Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. Endocrinology 1999; 140:814–817.


This important contribution shows that orexigenic peptides also modulate periph-eral components of the HPA axis, i.e. the adrenal steroid synthesis.


Carbohydrates

54 Andreis PG, Malenodwicz LK, Rebuffat P, et al. Galanin enhances corticosterone secretion from dispersed rat adrenal cortical cells through the activation of GAL-R1 and GAL-R2 receptors coupled to the adenylyl cyclase-depen-


66 Koylu EO, Balkan B, Kuhar MJ, et al. Cocaine- and amphetamine-regulated transcript (CART) and the stress response. Peptides 2006; 27:1956–1960. This comprehensive review paper specifically investigates the role of one anorexigenic neuropeptide (i.e. CART) with respect to its interactions with major stress systems.


76 Vulliemoz NR, Xiao E, Xia-Zhang L, et al. Melanocortin modulation of inflammatory cytokine and neuroendocrine responses to endotoxin in the monkey. Endocrinology 2006; 147:1878–1883. This describes one of the few recent experiments investigating the modulation of HPA axis responsiveness by anorexigenic neuropeptides. Importantly, by using an endotoxin the authors apply a central stimulus of the HPA axis.


