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ΤΙΤΛΟΣ ΔΙΠΛΩΜΑΤΙΚΗΣ ΕΡΓΑΣΙΑΣ

Links between HPA axis and adipokines

Σύνδεση μεταξύ υποθαλαμο-υποφυσιακο-επινεφριδιακού άξονα και αδιποκινών

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Θα ήθελα να ευχαριστήσω ιδιαιτέρως τον Καθηγητή κύριο Μαστοράκο Γεώργιο, τον Καθηγητή κύριο Σαλάκο Νικόλαο και την Καθηγήτρια κυρία Μπαργιώτα Αλεξάνδρα για την πολύτιμη βοήθεια τους στη συγγραφή αυτής της διπλωματικής εργασίας.

OUTLINE

ΤΙΤΛΟΣ

Σύνδεση μεταξύ υποθαλαμο-υποφυσιακο-επινεφριδιακού άξονα και αδιποκινών.

Περίληψη

Εισαγωγή: Στον ανθρώπινο οργανισμό, υπάρχει μια διαρκής αλληλεπίδραση μεταξύ του συστήματος του στρες(το οποίο περιλαμβάνει τον υποθάλαμο-υποφυσιακό- επινεφριδιακό άξονα) και του λιπώδους ιστού. Αυτή η αλληλεπίδραση διαμεσολαβείται από τις ορμόνες του ΥΥΕ άξονα όπως η κορτικοεκλυτίνη (CRH) , η αδρενοκορτικοτρόπος ορμόνη (ACTH) και τα γλυκοκορτικοειδή (GCs) και τις αδιποκίνες που παράγονται στον λιπώδη ιστό.

Ανασκόπηση: Στην παρούσα ανασκόπηση, παρουσιάζονται οι αμφίδρομες αλληλεπιδράσεις μεταξύ του ΥΥΕ άξονα και των περισσότερο μελετημένων αδιποκινών όπως η λεπτίνη και η αδιπονεκτίνη, όπως επίσης και των προ-φλεγμονωδών αδιποκυτοκινών, *παράγοντα* νέκρωσης όγκων (*TNF*) και ιντερλευκίνης (IL) 6. Επιπρόσθετα, οι αλληλεπιδράσεις αυτές περιγράφονται στην κανονικότητα όπως επίσης σε ειδικά κλινικά παραδείγματα διαταραχών σχετιζόμενων με το στρες όπως οι διατροφικές διαταραχές, η υποθαλαμική αμηνόρροια και η ενδογενής λειτουργική υπερκορτιζολαιμία σχετιζόμενη με καταστάσεις στρες. Παρουσιάζονται αντίστοιχα και οι νέες θεραπευτικές στρατηγικές που έχουν προκύψει στα ανωτέρω πεδία.

Συμπεράσματα : Κρίνεται απαραίτητη η επέκταση της έρευνας με στόχο την ανάδειξη των μηχανισμών που συμμετέχουν στην αλληλεπίδραση του ΥΥΕ άξονα και του λιπώδους ιστού. Η έρευνα θα πρέπει να εστιάσει ιδιαιτέρως στην ανάπτυξη νέων θεραπευτικών μέσων , στοχεύοντας στο δυσλειτουργικό λιπώδη ιστό σε διαταραχές που σχετίζονται με το στρες.

TITLE

Links between HPA axis and adipokines.

Abstract

Introduction: In the human organism, a constant interplay exists between the stress system [which includes the activity of the hypothalamic-pituitary-adrenal (HPA) axis] and the adipose tissue. This interplay is mediated by hormones of the HPA axis such as corticotropin-releasing-hormone (CRH), adrenocorticotropic hormone (ACTH) and glucocorticoids (GCs) and adipokines secreted by the adipose tissue.

Areas covered: In this critical review, the bi-directional interactions between HPA axis and the most studied adipokines such as leptin and adiponectin, as well as the pro-inflammatory adipocytokines tumor necrosis factor (TNF) and interleukin (IL) 6 are presented. Furthermore, these interactions are described in normalcy as well as in specific clinical paradigms of stress-related disorders such as eating disorders, hypothalamic amenorrhea and sress-related endogenous hypercortisolism states. Wherever new therapeutic strategies emerge, they are presented accordingly.

Expert commentary: Additional research is needed in order to clarify the mechanisms involved in the interplay between the HPA axis and the adipose tissue. Research should be focused, in particular, on the development of new therapeutic means targeting dysfunctional adipose tissue in stress-related situations.

Key words: adipokines, adiponectin, cortisol, CRH, cytokines, eating disorders, food intake, HPA axis, hypercortisolism, hypothalamic amenorrhea, IL-6, leptin, metreleptin, obesity, stress, TNF

1. Introduction

The survival of complex organisms depends on the maintenance of "homeostasis", a condition of dynamic equilibrium resulting from the adaptive responses of regulatory mechanisms to internal or external potentially threatening stimuli called "stressors". These regulatory adaptive mechanisms represent the "stress system" located both in the central nervous system and in peripheral organs [1]. The stress system is composed by the hypothalamic-pituitary-adrenal (HPA) axis and the locus ceruleus (LC)/norepinephrine (NE) system. The structures of the HPA axis are located in the paraventricular nuclei (PVN) of the hypothalamus (parvo- and magno-cellular neurons), the anterior lobe of the pituitary gland (basophilic cells) and the adrenal cortex [2]. The structures of the LC/NE system are located in the brain stem (LC), the sympathetic adrenomedullary cells and the sympathetic spinal ganglia and parasympathetic system. There is a constant interplay between the HPA axis and the adipose tissue. The latter secretes proteins called adipokines as well as pro-inflammatory adipocytokines that have both autocrine, paracrine and systemic (endocrine) action [3].

The aim of this critical review is to evaluate the bi-directional interactions between the HPA axis and the most studied adipokines such as leptin, adiponectin and the pro-inflammatory adipocytokines tumor necrosis factor (TNF) and interleukin (IL) 6 focusing on stress-related disorders. Subsequently, research studies regarding the complex interplay between the HPA axis and these adipokines in normalcy as well as in specific stress-related clinical disorders such as eating disorders, hypothalamic amenorrhea and stress-related endogenous hypercortisolism states are investigated. In addition, research targets regarding possible new therapeutic approaches in these stress-related clinical disorders are presented.

1.1. The Hypothalamic- Pituitary- Adrenal Axis and the stress response

The stress-induced activation of the HPA axis is initiated with the synthesis and release of corticotropin-releasing-hormone (CRH) from neurons in the medial parvocellular subdivision of the PVN of the hypothalamus, into the hypophysial- portal vascular system [4,5]. Other neurons in the same hypothalamic areas secrete another stress hormone, vasopressin (arginine vasopressin, AVP or antidiuretic hormone, ADH) which acts synergistically with CRH to stimulate ACTH secretion [6]. The PVN depends on inputs from other hypothalamic nuclei [such as the arcuate nucleus, the dorsomedial hypothalamic nucleus (DMH), and the preoptic area (POA)] and/or other

brain nuclei (such as the amygdala, the LC, etc.) which are integrated to positive stimulation of CRH secretion. On the other hand, the hippocampus and the prefrontal cortex suppress PVN activity and subsequently the activity of the HPA axis [4]. Consequently, CRH acts on the CRH-R1 receptors of the anterior pituitary corticotroph cells, stimulating the synthesis of proopiomelanocortin (POMC), the precursor peptide of adrenocorticotropic hormone (ACTH), as well as the release of stored ACTH [7]. The latter, when released into the systemic circulation, acts on the type 2 melanocortin receptor of the adrenal cortex and enhances the synthesis and release of glucocorticoids (GC) from the zona fasciculata [7]. Glucocorticoids are the peripheral effectors of the HPA axis and control the organism's adaptive response to the stressful situation *via* either intracellular-nuclear receptors such as the corticosteroid receptors type I or mineralocorticoid receptors (MRs), and corticosteroid receptors type II or glucocorticoid receptors (GRs) or *via* membrane-associated receptors [8]. The nuclear receptors initiate transcriptional activation or repression of different GC- regulated genes, while the membrane-bound receptors are coupled to downstream G protein-dependent signaling cascades which provide non-genomic effects [8]. Circulating GCs exert either positive (to the amygdala) or negative (to hypothalamus, pituitary gland and higher structures such as hippocampus) feedback *via* GRs in these tissues aiming at restoration of "homeostasis" (often as a new state of equilibrium so-called "allostasis") [1,2,7,9,10] (Figure 1).

Blood concentrations of GCs demonstrate a patent circadian rhythm in humans, with its zenith in early morning (active phase) and its nadir in late night (inactive phase). This rhythm is endogenously controlled by SCN *vi*a both HPA axis and direct sympathetic innervation, and by local molecular clocks in the adrenal cortex[11]. Chronic emotional stress in humans leads to blunted GC diurnal rhythm and elevated GC concentrations in the evening which in its turn leads to increased central obesity [11].

Interestingly, it has been recently demonstrated that there is a circadian fluctuation in the sensitivity of the GR *via* its acetylation, which attenuates its transcriptional activity [12]. GR acetylation, is increased in the morning and decreased in the evening, adversely to the physiological cortisol circadian rhythm fluctuation [12]. This phenomenon represents propably a local counter-regulatory mechanism that functions negatively in case of chronically stressed (or under frequent circadian shift) individuals whose mild evening cortisol elevations demonstrate enhanced activity on

target tissues resulting in an overall increased GC effect with subsequent metabolic implications [13].

On the other hand, a constant interaction exists between the HPA axis and the immune system. It involves pro-inflammatory cytokines such as TNF, IL-1, IL-6, IL-8, IL-12 and interferons (INF) α and β. These are glycoproteins secreted into the circulation by a variety of cells such as adipocytes, antigen-activated immune cells (T-cells, macrophages), neuron cells, fibroblasts and endothelial cells. These pro-inflammatory cytokines activate the HPA axis at multiple levels mainly *via* CRH and GCs stimulation and secretion [14,15]. More specifically, IL-6 is expressed in the supraoptic and PVN neurons and regulates the activity of both CRH and AVP genes, increasing CRH and AVP secretion by the corresponding PVN neurons [6]. In turn, endogenous or exogenously administered synthetic GCs suppress the secretion of these pro-inflammatory cytokines leading to control of the inflammatory chain reaction [1,4,7,16] (Figure 1).

1.2. Adipose tissue and adipokines

The adipose tissue forms a large organ widely dispersed in various sites of the body. It is involved in a variety of physiological activities while it consists of several types of cells such as pre-adipocytes, adipocytes (the predominant type of cells), macrophages, endothelial cells, fibroblasts and leucocytes. Except its major role which is energy storage, the adipose tissue functions also as an endocrine organ [3,17]. The population of macrophages in the adipose tissue augments significantly in parallel with its total mass, oscillating between 1% and 25% (in lean and obese subjects, respectively) [18]. It is distinguished histologically and functionally in white adipose tissue (WAT) which represents its major volume in the body, and brown adipose tissue (BAT). The former stores extra energy in form of triglycerides, their accumulation leading to obesity. Anatomically, WAT can further be distinguished into visceral (VAT) and subcutaneous (SAT) adipose tissue [19,20]. The former is located mainly around the internal organs of the abdominal cavity, and is associated with central obesity, insulin resistance, type 2 diabetes (T2DM), dyslipidemia, atherosclerosis and high mortality. Subcutaneous adipose tissue is rather accumulated in periphery and is associated with beneficial effects regarding the aforementioned metabolic risk factors [21]. Brown adipose tissue is inversely correlated to body mass index (BMI) and while is abundant in the newborn, in adults is decreased. It is located in the neck, the supraclavicular, paravertebral, mediastinal, para-aortic and suprarenal regions [22,23].

Interestingly, a sub-category of WAT consists of cells called *beige* or *brite* (brown-like-in-white) adipose cells which can function as BAT. These cells share common thermogenic characteristics with BAT and demonstrate an anti-obesity effect *via* fat consumption [17].

The endocrine functions of the adipose tissue are mediated by adipose tissue-derived proteins called adipokines (table 1) which are products of either the adipocyte cells or other type of cells harbored within the adipose tissue [3]. These bioactive factors have local (autocrine/paracrine) but also systemic (endocrine) actions. They communicate with other tissues in order to exert directly and/or indirectly pleiotropic functions, such as feeding behavior, glucose and lipid metabolism, inflammation and coagulation [3].

2. Adipose tissue, adipokines and the HPA axis

During the past three decades, a complex interplay has been revealed between the adipose tissue and the HPA axis [20]. Pro-inflammatory adipocytokines and adipokines secreted by the adipose tissue act directly and/or indirectly at all three distinct levels of the HPA axis (hypothalamus, pituitary, adrenals) (Figure 1). Indeed pro-inflammatory adipocytokines secreted by adipocytes and macrophages of the adipose tissue enhance HPA axis function acting directly and/or indirectly on the hypothalamic PVN, the pituitary and the adrenal glands, while GC inhibit their secretion (Figure 1) [14,15,24-29].

Of note, adipose tissue expresses both 11β-hydroxysteroid dehydrogenase type 1 and type 2 (11βHSD1 and 11βHSD2, respectively) enzymes. In humans, 11βHSD1 catalyzes the conversion of hormonally inactive cortisone to active cortisol, playing a crucial role in the GC metabolism, while 11βHSD2 acts inversely [30]. Adipocytes demonstrate low 11βHSD2 and relatively high 11βHSD1 activity [20]. In *in vivo* and *in vitro* studies in human obesity, 11βHSD1 activity has been found increased both in SAT and in VAT [31,32], leading, thus, to increased intra-adipose tissue cortisol with deleterious metabolic consequences [33]. In mice, there are data supporting the existence of an autocrine protective mechanism which down-regulates the adipose tissue 11βHSD1 in response to chronic high fat feeding. This mechanism disappears with worsening of obesity [31]. Pharmaceutical research is focused on therapeutic selective inhibition of 11βHSD1 aiming at attenuating the obesity-associated deleterious metabolic consequences, by reducing intra-adipose cortisol concentrations

without inducing systemic cortisol deficiency [31,34]. In human obesity, the majority of studies found circulating basal GCs concentrations increased while others found them unaffected. This discrepancy may be partially explained by the reduced plasma cortisol half-life in obesity due to increased urinary cortisol excretion[34-36]. However, HPA axis in obese subjects demonstrates loss of diurnal variation, increased responses to pharmaceutical stimulation (increased cortisol response to low ACTH test and to CRH-arginine vasopressin test) as well as resistance to low oral dexamethasone suppression test [36]. In fact, the obesity phenotype with excessive VAT, may represent, among other etiologies, a maladaptive response to chronic stress, with local and eventually systemic disregulation of the GC metabolism and the HPA axis activity [37].

Glucocorticoids acting *via* GRs (present in preponderance in VAT rather than SAT) and MRs (located in adipocytes) exert a dual action on adipose tissue. In one hand, GCs impair proliferation and promote differentiation of pre-adipocytes to mature adipocytes, while, on the other hand, they stimulate lipolysis in mature adipocytes, regulating thus, body expansion and distribution of the adipose tissue [37].

2.1. Leptin and the Hypothalamic-Pituitary-Adrenal axis

From the wide variety of adipokines, the most studied one in relation to the HPA axis is leptin. It is a 16kDa protein, product of the ob gene located on chromosome 6 and 7 in mice and humans, respectively, discovered in 1994. Its name derives from the greek adjective "leptos" meaning "thin", because of its anorexigenic, and therefore weight-reducing effects [9,38-40]. It is secreted mainly from the adipose tissue (but also in minimal quantities in mammary gland, stomach, skeletal muscle, placenta, pituitary gland and other brain structures [41]) in a pulsatile, circadian fashion, peaking in late evening in humans [11]. It is produced mainly in proportion to the amount of WAT (especially SAT) and in conditions of food excess. The circadian oscillations in leptin concentrations in human plasma and in cerebrospinal fluid are essential for its function and may play a critical role in metabolic regulations [11]. Leptin acts *via* leptin receptor (lepR) which is represented by at least 6 isoforms in many peripheral organs such as heart, liver, lung, ovaries, testes, endocrine pancreas, skeletal muscles, bones, hemopoietic organs and adipose tissue (local paracrine action), central nervous system (in choroid plexus, ventral tegmental area, ARC, PVN and DMH) pituitary and adrenal glands[9,42]. Leptin, acting in the hypothalamus, induces satiety and increases thermogenesis as well, contributing thus, to the maintenance of a negative energy

balance [43,44]. When found at low concentrations in fasting state or during weight loss, appetite increases and energy loss is limited [43,44]. Interestingly, in obesity increased concentrations of leptin suggest a state of leptin resistance resulting, eventually, from functional or (more rarely) genetically impaired receptor sensitivity [45].

Leptin, either secreted by the adipose tissue or locally secreted in the brain [41,44] interacts with the HPA axis. It seems that this interaction depends upon the level of HPA axis activation and circulating leptin concentrations. The information gathered as yet, regarding the various physiology aspects of this interaction should be interpreted with caution given that significant elements are missing in this field of physiology. The initial observations regarding the relationship of this adipokine with the HPA axis came from the homozygote (ob/ob) mutant Zucker rat which presents complete leptin deficiency and expresses a phenotype of obesity, hyperphagia, hyperglycemia and infertility. This rat presents with increased ACTH and corticosterone serum concentrations which decrease to normalcy following chronic administration of biosynthetic human leptin (subcutaneous administration of 100-300 μgr per rat per day for 30 days) [46-48]. In another animal model, the diabetic db/db mouse, which has mutated and dysfunctional lepRs and presents with an obese, diabetic phenotype and high concentrations of corticosterone [49], hypercorticosteronemia does not normalize after exogenous administration of leptin [48]. Thus, it appears that leptin exerts its direct and/or indirect effects on the HPA axis *via* functioning lepRs. In male Wistar rats, a bolus of 2 μg recombinant leptin administered intracerebroventricularly induced CRH secretion in a dose-dependent fashion while 2 injections of 3.5 μgr of recombinant human leptin to Long-Evans rats during a 40 h fast increased CRH in PVN by 38% [50,51]. The doses of 2 and 3.5 μg leptin are within the range of 10^{-9} M which is considered within the physiological range for circulating proteins. It has been suggested that this action participates in the central inhibition of food intake given that CRH is a potent anorexigenic hormone. In addition, leptin acting in the arcuate nucleus inhibits the expression of orexigenic peptides [neuropeptide Y (NPY) and agouti-related peptide (AgRP)] while it enhances the expression of anorexigenic peptides [proopiomelanocortin (POMC) and cocaine and amphetamine related transcript (CART)] leading to decreased food intake [52]. In humans with congenital leptin deficiency, on the other hand, 24-h urine cortisol concentrations are found within normalcy while cortisol is suppressed by 1 mg dexamethasone to less than 5 μg/dL [52-

56]. Leptin replacement in those patients increases mean 24-hour serum cortisol concentrations, altering the circadian rhythms of cortisol (decreased number but increased amplitude of pulses, increased morning peak) [56,57]. In humans with mutated lepR gene certain studies have found normal adrenal function [58]. Interestingly, in a class of patients with acquired hypoleptinemia, such as girls with hypothalamic amenorrhea (including anorexia nervosa), long-term (36 weeks) recombinant human leptin (r-metHuLeptin; metreleptin) administration, led to significantly reduced serum cortisol concentrations. Nevertheless, 24-h urine collection for free cortisol was not assessed in this study[59]. Further studies are needed to elucidate the role of leptin on human HPA axis when it is administrated to correct either its congenital deficiency (it regards morbidly obese patients) or its relative acquired deficiency (it regards abnormally low-weight patients).

Alternatively, in humans corticosteroids enhance leptin production and release by adipocytes *in vitro* [60-62] and *in vivo*, in normal-weight [63] and in obese subjects [64]. Specifically, administration of 2 mg dexamethasone twice daily resulted in elevated leptin concentrations at 08.00 am after 24 hours of treatment in healthy young men (mean age 24.9 years, mean BMI 24.4)[63]. In obese women (BMI 36.4±0.82 kg/m^2) but not in obese men (BMI 39.4±1.55kg/m^2) nor in normal weight subjects (BMI 22.1±0.41 kg/m^2), overnight administration of 1 mg dexamethasone induced plasma leptin elevation, indicating a probable greater sensitivity of female adipose tissue to GCs regarding leptin production [64].

Under *acute stress* (defined as the situation in which stress stimuli exert their noxious effect over a short period of time) conditions, endogenous leptin concentrations increase due to stress-induced hypercortisolemia (Figure 2A). In animal models, acute exposure to noise (1-3 hours), restraint, inmobilization (1-2 hours) or 10 min forced swimming, resulted to an increase of both corticosterone and leptin [65,66]. Interestingly, as well as when leptin is administrated acutely in pharmacological doses, HPA axis response to stressors is subsequently attenuated *via* leptin-associated inhibition of CRH release [65,67-70] (Figure 2A). When male mice were injected intraperitoneally (ip) with 2-4 μg/g body weight (BW) mouse leptin, ACTH and corticosterone plasma concentrations did not respond to restraint stress following treatment with the highest dose of mouse leptin (4 μg/g BW) indicating an inhibitory, direct and/or indirect, effect of leptin upon HPA axis activation. Moreover, CRH release in hemi-hypothalamic sections was found decreased in an injected-leptin dose-

dependent manner, following euthanization of mice In another study, immobilization-induced increases of plasma corticosterone in Wistar rats, were reversed by 0.1 and 0.5 mg/kg recombinant rat leptin injected ip [68]. Moreover, chronic (28 days) subcutaneous infusion of 6 μg/kg/day metreleptin in female rhesus monkeys, had no effect on basal cortisol secretion but attenuated cortisol and ACTH response to an acute HPA activation (injection of dexamethasone followed 18 h later by an injection of CRH). Of note, the administrated dose is lower than that observed in obese women and it reflects a state of positive energy balance[70]. It has been suggested that increased leptin concentrations enhance the negative feedback of GCs upon HPA axis *via* upregulation of the GR expression in the PVN and the hippocampus [71]. Indeed, a direct effect of leptin on the corticotrophs has been questioned and there is no ACTH response to leptin in cultures of rat pituitary cells *in vitro* [67]. Nevertheless, other evidence suggests that the intra-pituitary leptin production by other types of endocrine cells, such as somatotrophs and gonadotrophs, may act on corticotrophs in a paracrine fashion, serving in the integration of main physiological functions of the organism such as growth, reproduction and feeding [9,72]. Of note, lepRs are present in the anterior lobe of the pituitary on corticotroph cells [73] and in the adrenal cortex.

In a human model of acute stress (*acute perception of academic examination stress*) employed in a recent study with the help of a questionnaire derived from Hamilton Anxiety Scale in 85 healthy female students (aged 19–21 years; BMI 21.9 ± 1.6), serum cortisol and leptin concentrations were monitored at baseline (beginning of academic session) and on the day of the academic examination. Their concentrations increased between baseline and the examination day while those of cortisol correlated positively with the subjective perception of academic examination stress[74]. This leptin elevation could be attributed to stress-induced cortisol increase (Figure 2A). On the other hand, The direct inhibitory action of increased leptin concentrations on adrenal production of GCs has long been documented. When primary cultures of bovine adrenocortical cells were incubated with increasing concentrations (10-1,000 ng/ml) of recombinant mouse leptin for 24 h, ACTH-stimulated cortisol release was inhibited in a concentration-dependent manner[75]. In another *in vitro* study, isolated primary cells of human adrenal glands from kidney transplant donors were pre-incubated with metreleptin (10^{-10}-10^{-7} M) for 6 or 24 h and basal or ACTH-stimulated cortisol secretion was subsequently measured. Basal cortisol secretion was

unaffected by leptin, but a significant and dose-dependent inhibition of ACTH-stimulated cortisol secretion was observed [69].

In one study, the authors investigated the relationship between plasma leptin concentrations, hypothalamic opioid tone, and plasma ACTH secretory dynamics. Plasma leptin concentrations did not change after naloxone administration. However, there was a positive correlation between fasting, integrated plasma leptin concentrations, and plasma ACTH responses to naloxone. The correlation was stronger when leptin was normalized to BMI [76]. On the other hand, in a placebo-controlled study where centrally acting stimuli (naloxone, vasopressin, insulin-hypoglycemia test) of the HPA axis were administrated to eight healthy male volunteers (aged 27-51, BMI 23.3-30.1) and led to acute hypercortisolism, leptin concentrations did not increase following any of these stimuli [77]. These studies suggest that central activation of the HPA axis, doesn't seem to directly or acutely affect plasma leptin concentrations. However, plasma leptin concentrations may influence hypothalamic opioidergic tone and thus, modulate the magnitude of CRH release.

Under *chronic stress* (defined as the situation of continuous or repeated over time exposure to stressors) studied in animal models of *chronic mild stress* (depression-like behavior), rats exposed to various stressors over 3 weeks presented with hyperactivity of HPA axis and low leptin concentrations and low hypothalamic LepR mRNA expression [78]. Although leptin concentrations were greater in obese chronically stressed male rats than in normal-weight non-stressed and in normal-weight stressed rats, they were lower than in obese non-chronically stressed rats. In addition, an important down-regulation of LepRs was observed in the hippocampus and the hypothalamus of the obese chronically stressed rats resulting to depressive and anxiety-like behavior as well as cognitive impairment [79]. In this case the reduced leptin activity led to HPA axis hyperactivity [78,79] (Figure 2C). Furthermore, in healthy young men and women (mean age = 22.4years; BMI = 23.19 kg/m^2) treated with hydrocortisone the enhancing effect of GCs on leptin secretion was dose-dependent for the first 3-4 treatment days [80] while chronic administration of hydrocortisone (for 3 weeks) induced intolerance and had no effect on leptin concentrations in animal studies [81]. The anorexigenic effect of the relatively low leptin concentrations is diminished in chronic stress while at the same time other animal studies have shown upregulation of the orexigenic NPY system in relation to GCs excess, suggesting an explanation for stress-induced food intake and visceral obesity[82,83].

Of note, patients with Cushing syndrome present with elevated leptin concentrations in comparison with BMI-matched controls [84], suggesting that this effect is independent of the total body fat mass [85]. However, the hyperleptinemia in Cushing syndrome maintains its diurnal rhythm and persists after surgical treatment, indicating that the prolonged hypercortisolism doesn't affect leptin concentrations directly. Other factors such as body fat distribution and chronic hyperinsulinemia may play more important roles in leptin secretion in Cushing syndrome [86]. However, it is noteworthy that in food-dependent Cushing syndrome there is a paradoxical response of the adrenals to leptin resulting to cortisol secretion. This phenomenon could be explained by the presence of either aberrant, maybe mutated, LepRs or mutations involved in the secondary messenger cascade[87]. A direct effect of leptin on the corticotrophs has been questioned and there is no ACTH response to leptin in cultures of rat pituitary cells *in vitro* [67]. Nevertheless, other evidence suggests that the intra-pituitary leptin production by other types of endocrine cells, such as somatotrophs and gonadotrophs, may act on corticotrophs in a paracrine fashion, serving in the integration of main physiological functions of the organism such as growth, reproduction and feeding [9,72].

New therapeutic approaches have already emerged from this area of physiology. Research is focused on the use of leptin as an anti-stress, anti-anxiety, anti-depressive factor apart from the obvious interest in its anorexigenic potency. The only currently available pharmaceutical form of leptin is metreleptin. Metreleptin is approved by the FDA for the treatment of congenital or acquired generalized lipodystrophy (non-HIV related). For the rest of possible therapeutic indications, it is not yet approved because of lack of evidence of efficacy and/or safety. In obesity, metreleptin is only effective in the rare cases of congenital leptin deficiency. In common obesity, metreleptin is counterindicated and ineffective as these patients demonstrate both hyperleptinemia and central leptin resistance. To overcome central leptin resistance, research is focused on lepR sensitization methods [co-administration of leptin with anti-inflammatory or other weight-reducing drugs such as pramlintide (an amylin analogue)] [88].

2.2. Adiponectin and the Hypothalamic-Pituitary-Adrenal axis

Adiponectin, firstly described in 1995, is a 30-kDa protein, product of the apM1 gene, mainly synthesized and secreted by adipocytes [89]. Apart from adipose tissue,

adiponectin is found in tissues such as skeletal muscle, heart (cardiomyocytes), bone (osteoblasts), placenta, testis, ovary, liver, adrenal and pituitary glands [90]. Adiponectin is expressed mostly in SAT and is inversely correlated to the amount of visceral adiposity [91,92]. Its concentrations are reduced in obesity and lipodystrophy [93-95]. Peripheral administration of adiponectin decreases body weight and reduces visceral adiposity [96]. Three adiponectin receptors have been identified: the transmembrane adiponectin receptors (AdipoR) 1 and AdipoR2, which share a 66% homology in their amino acid sequence, and T-cadherin (CDH13). All three mediate its multiple metabolic actions in the tissues where they have been detected (liver, muscle, vascular wall, pancreas, adrenal gland, endometrium, testis, ovary, placenta, hypothalamus, pituitary, cortical and subcortical neurons of the brain, adipose tissue, macrophages and osteoblasts) [90,97,98]. Its main metabolic actions include insulin sensitizing, anti-inflammatory and anti-atherogenic actions. They are mediated by signaling pathways that include the adenosine monophosphate-activated kinase (AMPK), the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and the peroxisome proliferator-activated receptor alpha (PPARa) [99,100]. Low adiponectin concentrations have been associated with clinical components of the metabolic syndrome including negative association with insulin resistance [101], T2DM [102-104], inflammation, lipid concentrations [105,106], obesity [91,93], cardiovascular disease [107-109], gestational diabetes mellitus [110] and hypertension [92].

It seems that adiponectin is reciprocally associated to the HPA axis. In non-obese healthy men, the ultradian and circadian rhythm of adiponectin and cortisol share the same pattern, with a significant decline of both hormones at night, evoking a potential relationship between the two hormones [111]. Indeed, in human WAT, mRNA levels of adiponectin peak in the late morning [11]. Adiponectin mRNA expression was detected in rat adrenals where it enhances adrenocortical cell proliferation and stimulates corticosterone secretion in a dose-dependent manner [112]. Adiponectin can alter the expression of key genes involved in steroid synthesis. It affects directly steroid secretion in the adrenocortical cell line Y-1 while in human adrenocortical cells, it stimulates the production of cortisol *via* stimulation of the expression of steroidogenic acute regulatory (StAR) protein *via* the ERK1/2- and AMPK- dependent pathways [113]. Interestingly, short-term treatment with adiponectin seems to inhibit basal and ACTH-induced steroid secretion in mice [114]. Regarding the effects of HPA axis on

adiponectin expression, data from various studies are conflicting [115]. Some *in vitro* studies showed inhibition of adiponectin expression by GC administration [116-118], ACTH or stress-induced adrenal stimulation [112,119], while others did not show any GC-induced change in adiponectin concentrations [120,121]. Animal studies have shown also conflicting results. Intraperitoneal injections of hydrocortisone in both obese and non-obese rats lead to decreased adiponectin plasma concentrations [122] while decreased local intracellular GC action in adipocytes (produced by 11bHSD1 knockout or 11bHSD2 overexpression in transgenic mice) led to elevated adiponectin concentrations [123,124]. However, in other animal studies, a positive effect of administered GCs upon adiponectin concentrations was found [125,126]. In line with these results, adiponectin concentrations increased in clinical studies following GC administration either to healthy, non-diabetic humans [127,128], or to patients with IgA nephropathy [129] or to patients with renal transplantation [130]. Interestingly, 4 mg dexamethasone administered to T2DM patients and to healthy controls, inhibited AdipoR2 mRNA expression in nondiabetic subjects, while a small rise in plasma adiponectin concentrations was found [127]. In contrast, other studies demonstrated a negative effect of exogenous or endogenous GCs on the adiponectin concentrations of, respectively, healthy subjects or patients with Cushing syndrome [131]. The apparent discrepant conclusions in these studies could be attributed, among others, to the complex regulation of adiponectin by a large variety of distinct factors (transcription factors, signaling cascades and hormones) as well as the different response of the distinct types of adipose tissue (WAT, BAT, VAT, SAT) to these factors [132].

Adiponectin has been proposed as treatment for obesity and its complications due to its multiple beneficial metabolic functions (insulin-sensitizing, anti-inflammatory, anti-atherogenic). Oral administration of AdipoRon, an agonist of the adiponectin receptor 1 (AdipoR1), has shown promising results in improving diabetes in the obese rodent model db/db and it is under investigation for administration to humans [133]. However, unclear areas must be filled in till adipokine agonists or antagonists can be proven beneficial for the treatment of metabolic problems and dysfunctions of HPA axis and/or adipose tissue.

2.3. The pro-inflammatory adipocytokines tumor necrosis factor and interleukin-6 and the Hypothalamic-Pituitary-Adrenal axis

Tumor necrosis factor (TNF) is a potent pro-inflammatory cytokine of 157 aminoacids, produced mainly by activated monocytes and macrophages in tissues rich in these cells such as the adipose tissue, the hypothalamus, the pituitary gland, the ovary, the testis and the adrenal gland. Tumor necrosis factor concentrations are increased in obesity while they increase in response to stimuli such as tissue injury, infection and aseptic or septic inflammation. Its secretion depends also on local circadian clocks in immune cells, although a concrete circadian pattern is not proven in humans [11]. It is a main mediator of the immune response and inflammation while it plays an important role in the regulation of cell proliferation, differentiation and apoptosis. It acts *via* two receptors (TNF-R1 and TNF-R2), widely expressed in the body, including hypothalamus and anterior pituitary gland. Thus, TNF is involved in the cytokine-mediated communication between immune system, adipose tissue and HPA axis [134,135]. Tumor necrosis factor, interleukin (IL) 1β and IL-6, stimulate the HPA axis acting in the brain directly (by binding to cytokine receptors in the blood-brain barrier cerebral endothelium or by brain penetration *via* capillaries or by acting on CRH- and AVP- secreting neuron terminals at the median eminence through the fenestrated endothelia of the circumventricular organ) or indirectly, *via* action on peripheral ascending catecholaminergic neurons of the area postrema [14,15,24,25]. These cytokines stimulate CRH and AVP secretion, leading to increased ACTH production and finally, increased adrenal steroidogenesis [26,27]. In addition, TNF regulates directly steroidogenesis in the adrenal, the ovary and the testis (by upregulation of steroidogenic genes like StAR, 3β-HSD2, CYP17) [135] while it alters GR function. Thus, it affects the feedback control of GCs on the HPA axis [136]. By stimulating Iκb kinase, GCs lead to inhibition of Nfκb which in its turn inhibits TNF and the other pro-inflammatory cytokines production [29]. Nevertheless, this action was not demonstrated in patients with endogenous hypercortisolism. In effect, in patients with Cushing syndrome, TNF concentrations were in the normal range or inappropriately increased suggesting that in endogenous hypercortisolism, the inter-relationship between these two hormones finds a new level of homeostasis eventually through mechanisms such as down-regulation of receptors. This is due also to the excessive production of TNF by the increased amount of visceral fat in this pathologic entity [37,137]. Furthermore, a modification of GR which occurs following chronic

hypercortisolemia, leads to decreased ligand affinity and subsequently to decreased anti-inflammatory action of GCs [138,139]. Interestingly, patients with long-term cured Cushing syndrome present with increased concentrations of TNF and other inflammatory cytokines, maintaining a state of chronic "low grade inflammation" [137].

Interleukin 6 is another pleiotropic cytokine produced and secreted by a variety of cells, including adipocytes, macrophages, fibroblasts and endothelial cells. It is also expressed in immune tissues, hypothalamus, anterior pituitary and adrenal cortex [140]. Interleukin 6, like TNF, is expressed more in VAT than SAT [141] and demonstrates local autocrine/paracrine action in the adipose tissue as well as systemic action, promoting coagulation and pro-inflammatory reaction. This cytokine is secreted in humans in a circadian rhythm, with a zenith in the early morning[142,143]. Its secretion increases in case of disturbances of the homeostasis such as tissue injury, septic (as in sepsis) or aseptic (as in rheumatoid arthritis) inflammation and in any type of physical or psychological stress such as exercise [5,29,140,144].

Moreover, IL-6 plasma concentrations are significantly increased in obesity and decreased after weight loss [145]. It has been estimated that one third of total circulating concentrations of IL-6 originate from the adipose tissue. Thus, obesity represents a state of low-grade inflammation [146]. Interleukin 6 stimulates HPA axis *via* CRH and AVP neurons in the hypothalamus [144]. Indeed, in the past we have shown that administration of recombinant IL-6 to humans led to substantial increase of the AVP, ACTH and GC peripheral concentrations [15,143,147,148]. Furthermore, IL-6 may regulate steroidogenesis locally in the adrenals as it has been shown *in vitro* [5]. In turn, GCs inhibit IL-6 secreted by the cells of the immune system [28]. The tonic negative feedback loop between endogenous cortisol and IL-6 is shown in patients with Cushing syndrome who present with low concentrations of IL-6. In the immediate postoperative period, when these patients become hypoadrenal, a significant increase of IL-6 is observed, while its concentrations decrease again after GC replacement therapy [149].

With regard to insulin resistance, low-grade inflammation is associated with its pathogenesis particularly of that attributed to visceral obesity [150,151]. Tumor necrosis factor is secreted by the increased number of macrophages in the adipose tissue of obese subjects and it contributes to insulin resistance while it decreases following weight reduction [152]. Circulating TNF concentrations are higher in visceral obesity compared with peripheral obesity [151]. In the investigation of the underlying mechanisms, initial studies suggested an impairment of insulin receptor (IR) which

exerts a tyrosine kinase activity [153,154]. Accordingly, TNF administration in skeletal muscle *in vitro* impaired insulin stimulation of glucose uptake by the cells and GLUT4 translocation to plasma membrane [155]. The defect in insulin signaling was attributed to TNF-induced serine phosphorylation of IR and IR substrate (IRS) 1, impairing thus insulin-stimulated autophosphorylation of IR on multiple tyrosine residues and subsequent tyrosine phosphorylation of IRS-1 and leading to insulin resistance [153-155]. At the same time, the role of IL6 in the pathogenesis of insulin resistance remains controversial, with studies supporting the hypothesis that IL6 impairs insulin sensitivity [156,157] and others that IL6 enhances both insulin function [158] and leptin action [159]. Recent research indicates that IL6 and leptin share a common intracellular signal transduction pathway downstream of their respective receptor systems, glycoprotein 130 receptor β (gp130Rβ) and LepRb. Interestingly, beyond leptin analogs, gp130R agonists have been suggested as possible anti-obesity therapeutic options [160].

Both of these pro-inflammatory cytokines demonstrate crucial functional characteristics regarding both the stimulation of the HPA axis and the development of insulin resistance in humans, which makes them interesting targets for pleiotropic pharmacologic interventions.

3. Adipokines and the Hypothalamic-Pituitary-Adrenal axis in stress-related disorders

3.1. Food intake and eating disorders

3.1.1 Food intake and the Hypothalamic-Pituitary-Adrenal axis

Feeding behavior is regulated by a complex interaction between neural and endocrine systems. The afferent peripheral system, includes a variety of hormones such as insulin (secreted by the endocrine pancreas in response to a meal), leptin, ghrelin (an orexigen produced by the gastrointestinal tract in absence of food intake) and gut hormones (they provide satiety signals) such as glucagon-like peptide 1 and cholecystokinin. These signals are interpreted by various brain centers such as the nucleus tractus solitarius (NTS) and the CNS food intake integrating unit, located in the ventromedial hypothalamus (VMH), the lateral hypothalamus (LHA) and PVN. Then, efferent signals from these brain centers, in particular neuropeptides, stimulate either the sympathetic nervous system to induce energy expenditure or the parasympathetic nervous system to increase insulin secretion which promotes adipogenesis and energy

storage. Energy expenditure is mediated by the activation of β3 adrenergic receptors in peripheral adipose tissue *via* induction of thermogenesis and lipolysis [43]. The neuropeptides implicated are either anorexigenic (POMC, CART and CRH) or orexigenic (NPY, AgRP and MCH). Corticotropin-releasing hormone is a potent anorexigenic neuropeptide. Direct administration of CRH into PVN inhibits nighttime and fasting-induced food intake. This hormone also induces energy expenditure *via* stimulation of thermogenesis and lipolysis [161].

In addition internal circadian function interferes with feeding behavior. The former synchronizes constantly with external environmental factors (i.e. light, food intake) in order to coordinate diverse biological processes, such as sleep-wake rhythms, eating, energy metabolism and immune function [162]. Apart from the HPA axis, studies have recently shown that adipokines (i.e. leptin, adiponectin) and pro-inflammatory cytokines (i.e. TNF, IL-6) demonstrate also circadian rhythms [162]. However, normal circadian physiology is threatened by exogenous factors such as nocturnal light exposure, shift work and social jet lag (lack of sleep during workdays and excess of sleep during free days) [163]. The disruption of circadian physiology has been linked to serious health problems. Obesity (in particular VAT increase), cancer, cardiovascular disease, depression, autoimmune diseases and insulin resistance-associated situations (i.e. T2DM) are linked to the disruption of the diurnal physiological rhythms of the HPA axis and the adipokines [162,164-166].

3.1.2. Eating disorders and the Hypothalamic-Pituitary-Adrenal axis

Eating disorders are characterized by disturbances in feeding behavior and by pathological cognition of one's body shape and weight. Accordingly, there are three main eating disorders: anorexia nervosa (AN), bulimia nervosa (BN) and binge eating disorder (BED). The latter is characterized by recurrent uncontrolled binges with absence of purging or other compensatory behaviors [167]. Hypothalamic-pituitary-adrenal axis seems to be activated in AN and BN, as elevated cortisol is detected in both syndromes [167,168] while cortisol suppression by exogenous GCs is impaired [169]. In BED, studies examining cortisol concentrations led to conflicting results. In obese subjects with BED, 24-h urinary free cortisol concentrations seem to be inversely correlated with the severity of the disease insinuating conditions typically linked to low HPA axis activity such as mood and anxiety disorders [170,171]. In addition results of another study imply chronic down-regulation of the HPA axis in patients with BED. Interestingly, acute activation of the axis is associated with feelings of stress and food

craving in these subjects [172]. This can probably suggest poor response to satiety signals while being under a stressful situation [173]. As it has been long observed in healthy individuals, in acute stress, the activation of the HPA axis inhibits food consumption, probably due to the anorexigenic effect of CRH [43]. Conversely, in case of chronic stress, the sustained activation of the HPA axis leads to high circulating cortisol concentrations and induces calorically dense food intake *via* diverse hormonal interferences including insulin, NPY, CRH, leptin etc.). Indeed, high cortisol concentrations lead to insulin and leptin resistance while they stimulate NPY release *via* inhibition of CRH, all contributing to increased food intake (stress eating) [174]. As for leptin concentrations, most studies have detected low concentrations in AN and BN in basal and postprandial measurements. In BED leptin concentrations seem to be higher in obese subjects than in normal weight controls [167].

3.2. Functional hypothalamic amenorrhea

Functional hypothalamic amenorrhea (FHA) is a condition of chronic anovulation with no apparent anatomic or organic cause. It is usually a reversible form of amenorrhea which is often associated with stressful situations, psychological distress, excessive exercise, energy deficit (combined with weight loss or not) or chronic diseases. The diagnosis of FHA is a diagnosis of exclusion. The cause of anovulation is a functional reduction in GnRH drive, which manifests as reduced LH pulse frequency. Subsequently, LH and FSH concentrations are insufficient to maintain full folliculogenesis and ovulatory ovarian function [175].

In FHA, the critical factor is energy deficit (independently of changes in body weight). Indeed, LH pulsatility is disrupted even in women with normal body composition and regular menstruation when energy availability is restricted below 30 kcal/kg of lean body mass (LBM) [176]. Energy deprivation states encountered in eating disorders, especially anorexia nervosa, chronic diseases and elite athletes, are characterized by undernutrition, low body fat mass, activated HPA axis (increased CRH, ACTH and cortisol concentrations), increased adiponectin and low leptin, IGF-1 and T3 concentrations [175,177-179]. In FHA, leptin concentrations are significantly decreased reflecting the low body fat percentage or restrictive eating behavior or a combination of both. Interestingly, amenorrheic women present higher incidence of abnormal eating, particularly bulimic type behavior, and present with lower leptin

concentrations and higher cortisol concentrations, independently of body fat [177]. This fact underlies the importance of restrictive eating behavior in the pathophysiology of FHA. There is also a loss of the circadian rhythm of leptin concentrations in FHA [178].

Adiponectin, in contrast to leptin, is elevated in energy deprivation states. In young women (18 - 35 years old; BMI 21.5±0.4 kg/m^2), adiponectin concentrations were significantly elevated in those exercising and presenting with FHA compared to those exercising or being sedentary and presenting with ovulatory cycles [180]. A role for adiponectin in the reproductive function has been suggested including modulation of steroidogenesis within granulosa and thecal cells but pathophysiological mechanisms remain still unclear [181,182].

There is a tight link between activation of HPA axis and reduction in GnRH drive in women with FHA [175]. Indeed, these women demonstrate higher concentrations of serum 24-hour cortisol when compared to controls, similar to women with eating disorders. Interestingly, females with FHA demonstrate higher prevalence of disordered eating behavior such as dieting, bulimia and high food preoccupation [177]. Moreover, in young athletes with amenorrhea, there are reported higher nighttime cortisol concentrations than in eumenorrheic athletes and nonathletes [179]. Hypothalamic-pituitary-adrenal axis inhibits the hypothalamic-pituitary-ovarian (HPO) axis in multiple ways and levels [183].

Leptin stimulates GnRH neurons indirectly as there are no LepR in these cells. LepR expression is also limited in kisspeptin neurons but is abundant in interneurons in the ventral premamillary nucleus (PMv), in the median preoptic area (MPO) and in the ARH (NPY/AgRP and POMC/CART cells). These interneurons are necessary to transfer leptin signal to GnRH neurons. Leptin stimulates reproductive system through inhibition of the HPA axis, the POMC neuronal system and NPY secretion (in women with negative energy balance) and *via* activation of the LC/NE system [184]. Consequently, leptin concentration can modulate the onset of puberty and reproduction *via* indirect stimulation of GnRH neurons. [185].

There are a few studies that have evaluated the effect of leptin administration in FHA. In one prospective study, the administration of metreleptin in women with FHA led to increase of the ovulatory cycles and significantly increased concentration of LH, estradiol, IGF-1, thyroid hormone and bone-formation markers than in controls [186]. In another randomized, double-blinded, placebo-controlled trial, metreleptin

administration for more than 36 weeks in women with FHA resulted in restoration of menses (>50% ovulatory) and increased concentrations of estradiol, progesterone, IGF-1, fT3 and osteocalcin concentrations while cortisol concentrations decreased [59]. These data indicate an important role of hypoleptinemia in the pathophysiology of the reproductive, neuroendocrine and bone abnormalities in FHA [59].

3.3 Stress-related endogenous hypercortisolism states

A high proportion of patients who present overt visceral obesity associated with insulin resistance and other signs of the metabolic syndrome, suffer a wide range of psychological manifestations including melancholic depression and anxiety, demonstrating frequently a perception of "uncontrollable" stress [187]. These chronically stressed obese patients usually present with mild endogenous hypercortisolism. These patients present a blunted cortisol circadian rhythm (due to evening nadir elevations and morning zenith decreases), along with augmented cortisol elevation in response to lunch and impaired cortisol suppression by low dose overnight dexamethasone test [188]. This state of chronic stress- and obesity- associated mild hypercortisolism leads to a "pseudo-Cushing" state that has to be differetiated from frank Cushing syndrome. It is long suspected that this stress- and obesity- associated chronic adrenal stimulation might be associated with the development of non-functioning adrenal incidentalomas (NFAI)[189]. The latter, an "endocrine epidemia" of nowadays, were considered in the past as hormonally inactive tumors of the adrenals. Nevertheless in many cases, subclinical hormonal activity of the cortex or medulla is justified. Subsequently, the initial hypothesis of stress- and obesity- associated functional hypercortisolemia in relation to the development of incidentalomas is transformed to *a-hen-and-egg* enigma because the subclinical hormonal activity of incidentalomas (whenever manifesting) results in co-existence with metabolic diseases (subclinical or clinical Cushing syndrome, pheocromocytoma, obesity, hypertension, DM type 2) [189,190]. Of note, a recent study aimed to reveal associations between the hormonal activity of NFAI and possible oversecretion of adipokines and pro-inflammatory adipocytokines in relation to the pathophysiology of the associated metabolic syndrome [191]. In this study, 18 patients (aged 25–66 years, mean: 54.11 ± 11.78; BMI 27.00 ± 4.24 kg/m^2) with adrenal incidentalomas without known hormonal activity were compared to 18 healthy volunteers. Patients with NFAI presented with significantly higher concentrations of TNF and IL6 and significantly lower concentrations of adiponectin, which did not correlate with excess cortisol or

catecholamine secretion [191]. Six months after surgical removal of the incidentalomas in 5 patients, the concentrations of IL6 and leptin decreased while adiponectin concentrations increased in all studied cases. The authors suggested that low adiponectin concentrations may play a crucial role in the link between NFAI and cardiovascular disease. In accordance to that, other studies have demonstrated that even mild chronic subclinical hypercortisolemia of seemingly NFAI may lead to decreased adiponectin concentrations and predispose to metabolic diseases [192].

3.4. HPA axis and lipodystrophy in human immunodeficiency virus (HIV)-infected patients

It is suggested that HIV infection is a model of ´´chronic stress´´ and affects HPA axis *via* several pathways, leading to a wide spectrum of abnormalities, from adrenal insufficiency to symptomatic hypercortisolemia. Treatment of HIV infected patients with combined antiretroviral therapy (cART) can also induce hypercortisolemia [7] and lipodystrophy. The latter is a medical condition characterized by redistribution of the adipose tissue with lipoatrophy (loss of SAT) in extremities and face and increase of dorsocervical adipose tissue (buffalo hump) and VAT [7, 195]. Since the phenotype of lipodystrophy closely resembles the phenotype of chronic GC excess, as seen in Cushing syndrome, the term pseudo-Cushing was initially used to describe this syndrome. Lipodystrophy in HIV patients is multifactorial. Pathogenetic factors might be related to HIV infection itself, to the host (visceral obesity and chronic inflammation) and to the treatment. It is usually associated to metabolic syndrome with mixed hyperlipidemia and insulin resistance partially related to metabolic dysfunction of the adipose tissue. In effect, there is evidence of increased adipose tissue macrophage infiltration and a shift in adipokine balance with significantly reduced levels of adiponectin and leptin, and increased levels of proinflammatory cytokines such as TNF, IL-6 and IL-1 in both adipose tissue and plasma [195, 196]. The high levels of inflammatory cytokines stimulate the HPA axis but also provoke over-expression of the enzyme 11-βHSD1 in the liver and adipose tissue, leading to high local intra-adipose and peripheral cortisol [7].

4. Summary

In summary, there is a constant bi-directional interplay between HPA axis and adipose tissue mediated in one hand by hormones, mainly CRH, ACTH and GCs and on the other hand by adipokines and pro-inflammatory adipocytokines. The importance of this interplay is unraveled in diverse clinical situations related to stress-related disorders such as eating disorders, hypothalamic amenorrhea and stress-related endogenous hypercortisolism.

Regarding the investigation of this interplay, leptin comes first among the most studied adipokines. Absolute leptin deficiency (ob/ob Zucker rat, congenital leptin deficiency) leads to enhanced HPA axis activity, a situation which in many cases is reversed with leptin treatment [39,46-48,193]. In addition, in high pharmacological doses or under stressful situations, leptin attenuates HPA axis in many different ways. First, by reducing CRH release [65,67-70], second by upregulating GR expression in the PVN (enhancement of the GCs negative feedback on the HPA axis) [71] and third, by direct inhibitory action on adrenal glands [75]. In chronic stress, there is a down-regulation of leptin/LepR signaling in hypothalamus which leads to enhancement of the HPA axis activity [78,79]. Of note, GCs (either endogenous or exogenous) enhance leptin production from the adipose tissue[60-64], although resistance in this action is developed after prolonged hypercortisolism [81,86]. In states of leptin excess such as obesity or T2DM, a state of leptin tolerance is developed [45] with subsequent loss of its anorexigenic action. During stress, the activation of the HPA axis is involved in the feeding response. While CRH is anorexigenic, GCs may be orexigenic or anorexigenic depending on whether they activate receptors in the hypothalamus (orexigenic action by increasing expression of NPY and decreasing expression of CRH) or in adipocytes (anorexigenic action by enhancing the release of leptin) [65].

Adiponectin is another important adipokine because of its insulin sensitizing, anti-inflammatory and anti-atherogenic actions [99,100]. Its concentrations are reduced in obesity a situation linked to HPA axis dysregulation and many metabolic abnormalities [93-95]. However, investigation on the reciprocal relationship of adiponectin and HPA axis has led to controversial results and the field remains open.

Another category of adipokines, the pro-inflammatory adipocytokines (TNF, IL-1, IL-6) have a well-studied stimulatory effect upon multiple levels of the HPA axis. Many stressors lead to increased concentrations of these pro-inflammatory

adipocytokines. The latter are also elevated in obesity [145] while their production by immune cells is inhibited by GCs [14,15,24-29].

In conclusion, a challenging field regarding the interplay between HPA axis activity (particularly during stress-related situations) and adipokines remains to be investigated in depth. The mechanisms of physiology involved should be uncovered and described in more detail. The development of new therapeutic approaches might target a wide variety of medical problems related to dysfunctional stress response in relation to food consumption, energy expenditure and finally the adipose tissue itself.

5. Expert commentary

The interaction between HPA axis and adipokines is an important part of a research field covering the relationship between the stress response and the adipose tissue. The importance of this interaction has attracted attention in the last decades, due to the augmented concern regarding increasing health problems such as metabolic syndrome, obesity, eating disorders, depression, mood and food disorders related to chronic stress (either physical or emotional). In this context, adipokines seem to play a crucial role. They are involved in the regulation not only of the HPA axis but also of other endocrine axes. New therapeutic approaches intent to take advantage of the beneficial effects of the adipocytokines aiming to a better metabolic regulation. Metreleptin, the only currently available and approved by the FDA, pharmaceutical form of leptin, is already used for the treatment of congenital or acquired generalized lipodystrophy (non-HIV related) and remains under investigation with hopeful results for its use as an anti-stress, anti-anxiety, anti-depressive factor apart from the obvious interest in its anorexigenic potency. In addition, studies investigating the effect of metreleptin administration in women with FHA have led to positive results with restoration of menses, increase of ovulatory cycles and improvement of the hormonal profil. Moreover, adiponectin has been proposed as treatment for obesity and its complications due to its multiple beneficial metabolic functions (insulin-sensitizing, anti-inflammatory, anti-atherogenic). Oral administration of AdipoRon, an agonist of the adiponectin receptor 1 (AdipoR1), is already under investigation for administration to humans after successful animal studies. However, unclear areas must be investigated till adipokine agonists or antagonists can be proven beneficial for the treatment of metabolic problems and dysfunctions of HPA axis and/or adipose tissue.

In conclusion, there is a need for better understanding of the physiology of

adipokines in order to elucidate all their connections with the HPA and the other endocrine axes either in normal physiological states or in pathologic (eventually stress-related) states.

6. Five-years view

Along the next five years there are a lot of challenges and new prerspectives regarding the reciprocal relation between the HPA axis and the adipokines. Research should extent the current knowledge on the field of pathophysiology of HPA axis response to the stress in relation to the adipose tissue. This knowledge will set the background for the investigation of new therapeutic approaches in stress related diseases. Nevertheless, new therapies can be investigated under condition that new standardized methods of evaluation of stress intensity (including biochemical markers of the HPA axis) will be established. In addition, it is necessary to conceive more accurate methods of measurement of the peripheral concentrations of the main adipokines such as leptin and adiponectin and of the adipocytokines TNF and IL6 in order to use them as disease markers of stress related conditions in everyday practice. Stress-related disorders present a high spread nowadays and there is a need for holistic diagnostic and therapeutic approaches including the obscure as yet communication of the adipose tissue with stress.

Key issues:

- There is a bi-directional interplay between stress reponse and adipose tissue. Hypothalamic-pituitary-adrenal axis interacts reciprocally with the adipose tissue, *via* hormones such as CRH, ACTH, GCs and adipokines such as leptin, adiponectin and proinflammatory adipocytokines.
- Leptin interacts with the HPA axis in a complex way contributing to attenuation of the HPA axis activity in acute stress situations.
- In chronic stress, there is a down-regulation of leptin/LepR signaling in hypothalamus which leads to enhancement of the HPA axis activity.
- In obesity, as well as in sustained HPA axis activation, a state of leptin resistance develops due to impaired leptin receptor sensitivity (functional hypoleptinemia).

- Adiponectin has reciprocal relationship with the HPA axis, altering the expression of key genes involved in steroid synthesis.
- Pro-inflammatory adipocytokines mediate the communication between the immune system, the adipose tissue and the stress system, regulating the HPA axis centrally (in the brain) but also peripherally (direct effect on steroidogenesis).
- Excessive, defective or dysfunctional adipose tissue is related to dysfunctional HPA axis and stress response.

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Table 1. Most studied adipokines, as included in the following reviews [194,195].

Leptin
Adiponectin
Resistin
Chemerin
Apelin
Interleukin-6 (IL-6)
Interleukin-1β (IL-1β)
Interleukin-10 (IL-10)
Visfatin
Omentin
Vaspin
Plasminogen activator inhibitor 1 (PAI-1)
Monocyte chemoattractant protein 1 (MCP1)
Retinol binding protein 4 (RBP4)
Tumor necrosis factor (TNF)
Progranulin
Complement C1q tumor necrosis factor-related protein 4 (CTRP-4)
Transforming growth factor β (TGFβ)
Secreted frizzled-related protein 5 (SFRP5)
Fibroblast growth factor 21 (FGF 21)
Angiopoietin-like protein 2
Bone morphogenetic proteins (BMPs)
Cathepsins
Lipocalin
Nesfatin- 1

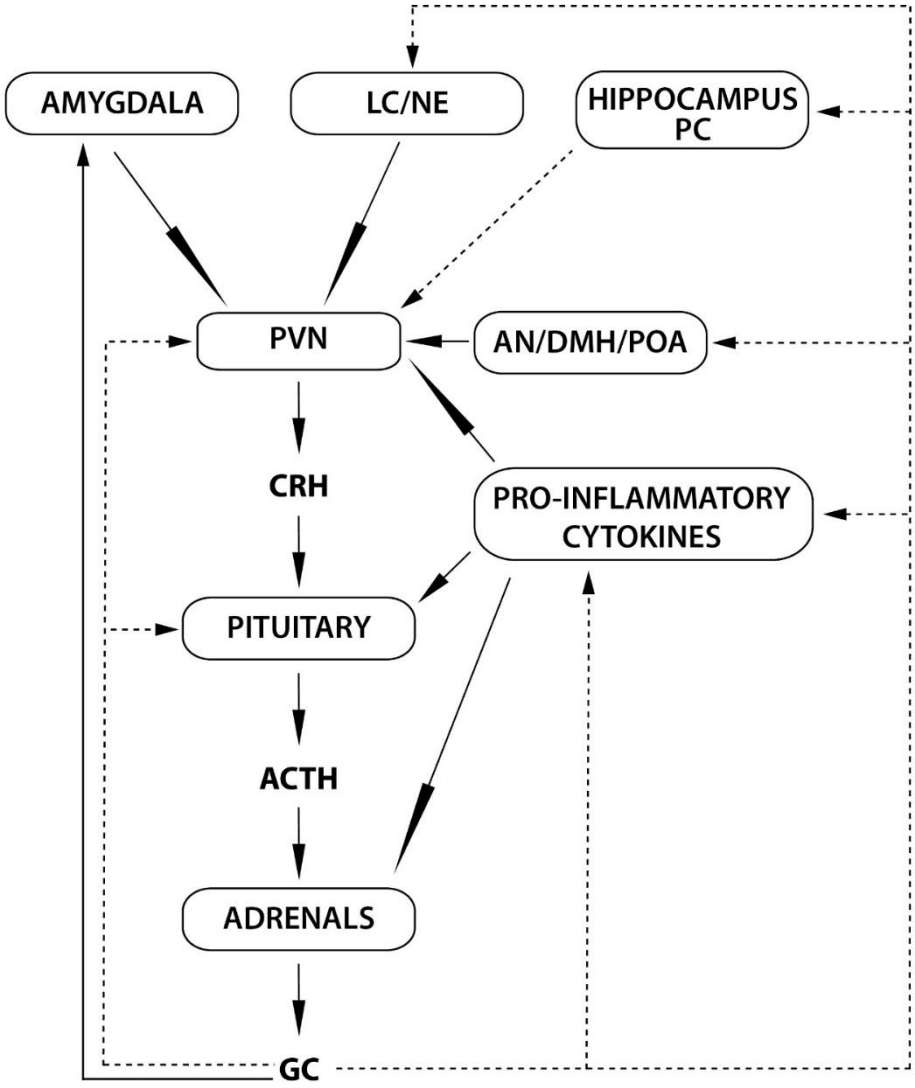

Figure 1: Immune/inflammatory and neuronal regulation of HPA axis.

The activation of the immune/inflammatory system leads to secretion of proinflammatory cytokines that activate the HPA axis at multiple levels. Various brain nuclei contribute to the activation of the HPA axis [amygdala, locus caeruleus/norepinephrine (LC/NE), dorsomedial hypothalamic nucleus (DMH), arcuate nucleus (AN), preoptic area (POA)] while others [hippocampus and prefrontal cortex (PC)] to its inhibition. In turn, glucocorticoids activate amygdala and inhibit LC/NE, AN, DMH, POA, hippocampus and PC.

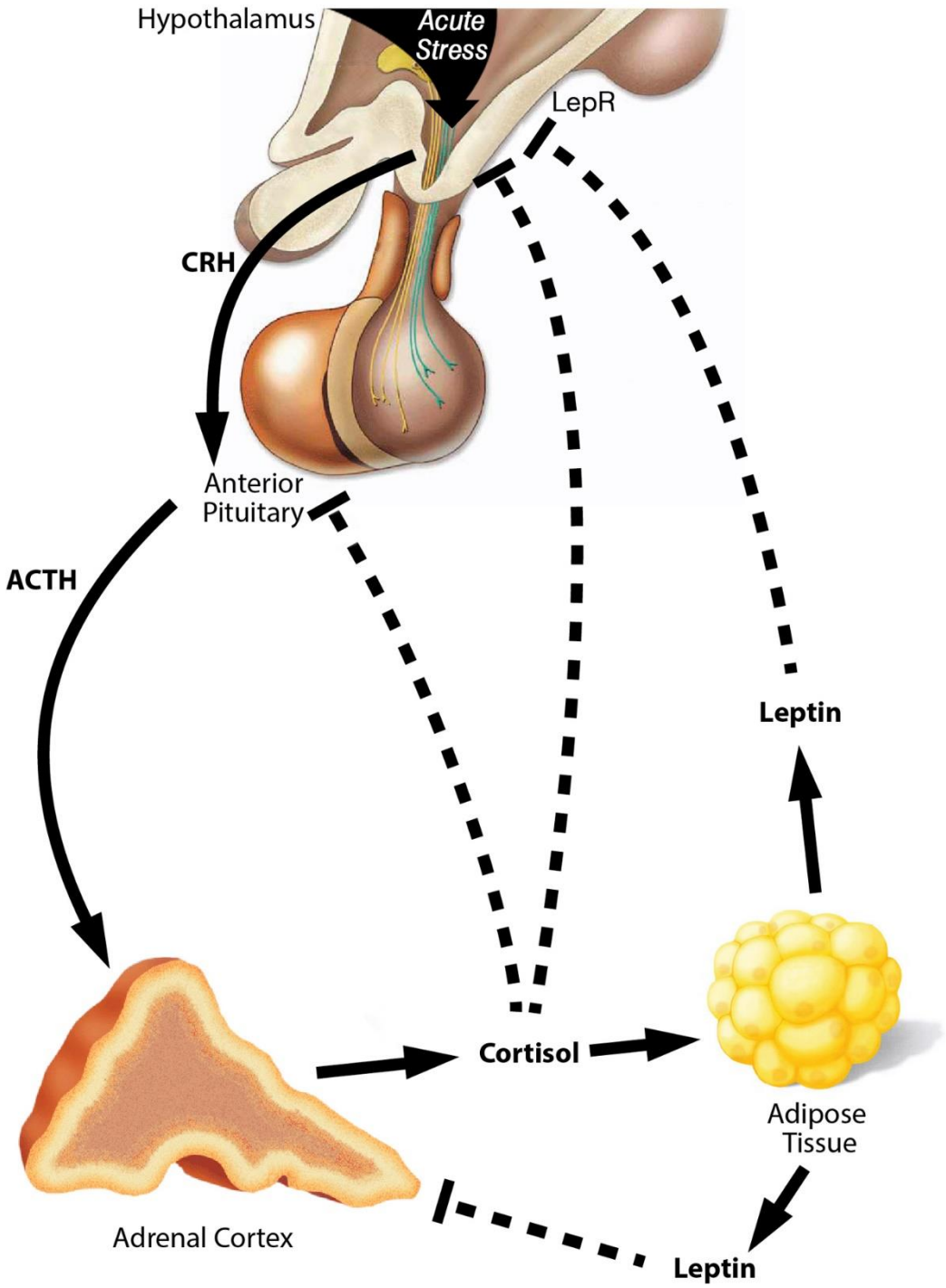

Figure 2A: The HPA axis-Leptin interplay in acute stress.

Acute stress leads to elevated leptin secretion via increased secretion of cortisol. Leptin, in turn, attenuates HPA axis response to stress. This, added to the inhibitory feedback of glucocorticoids over the HPA axis, points at the return of glucocorticoid secretion to a basal state.

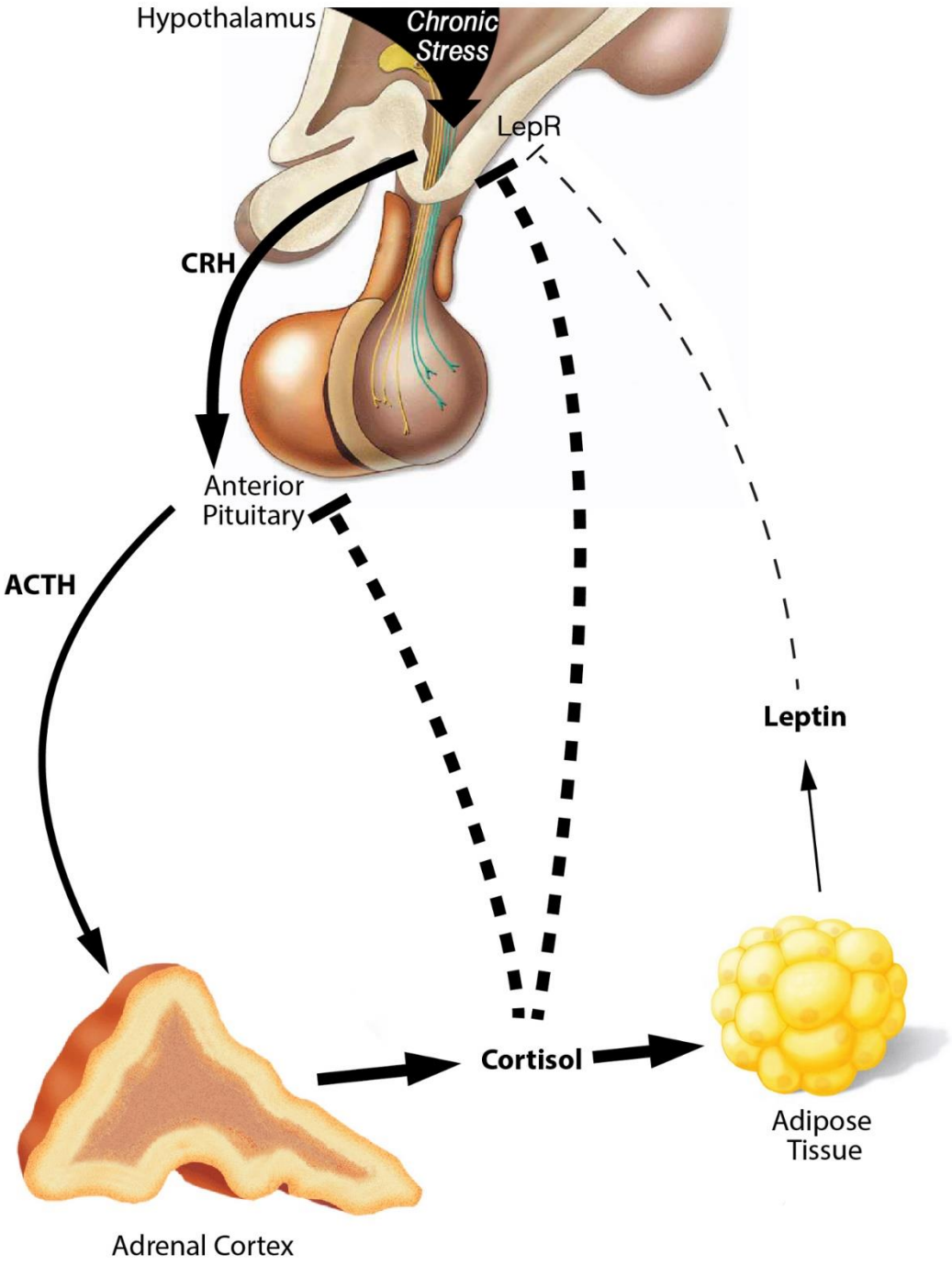

Figure 2B: The HPA axis-Leptin interplay in chronic stress.

In chronic stress, there is a down-regulation of leptin/LepR signaling in hypothalamus which leads to enhancement of the HPA axis activity.

(Solid lines: positive correlation, Dotted lines: negative correlation)