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Adipokines and Cardiovascular Risk in Cushing's Syndrome

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Abstract
Cushing's syndrome (CS) is associated with increased cardiovascular morbidity and mortality. Recent evidence also suggests that increased cardiovascular risk may persist even after long-term remission of CS. Increased central obesity, a typical feature of CS, is associated with altered production of adipokines, which contributes to the pathogenesis of several metabolic and cardiovascular complications observed in this condition. In vitro and in vivo studies have shown a relationship between cortisol and adipokines in several experimental settings. In patients with either active or 'cured' CS, an increase in leptin and resistin levels as well as the release of pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6, may be associated with increased cardiovascular risk. For other adipokines, including adiponectin, results are inconclusive. Studies are needed to further elucidate the interactions between clinical and subclinical increases in cortisol production and altered adipokine release in CS.

Introduction

Endogenous Cushing's syndrome (CS) is a consequence of chronic exposure to high circulating levels of glucocorticoids. Cortisol is an end product of the hypothalamic-pituitary-adrenal (HPA) axis, and is produced by the adrenal cortex in response to pituitary adrenocorticotropin (ACTH). ACTH in turn is regulated by hypothalamic secretion of corticotropin-releasing hormone (CRH) and vasopressin. CRH, vasopressin and ACTH secretion is controlled by cortisol levels through a negative feedback mechanism at the level of the hypothalamus and pituitary. In healthy individuals, cortisol is secreted in a circadian rhythm; levels peak in the morning and then decrease during the day to a nadir around midnight. Endogenous CS is characterized by loss of this circadian rhythm, together with impaired physiologic feedback within the HPA axis. CS is usually caused by excessive ACTH release from a pituitary corticotrope adenoma (Cushing's disease, CD) and less frequently by ectopic ACTH or (very rarely) CRH production. CS can also be ACTH-independent when caused by increased cortisol secretion by adrenocortical tumors or hyperplasia [1].

Endogenous hypercortisolism is associated with increased cardiovascular risk as well as a number of comor-

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bidities, and in untreated patients, results in a 4 times higher mortality rate than expected in the normal population [2].

Chronic glucocorticoid excess in CS is commonly associated with a cluster of cardiovascular risk factors that strongly impact morbidity and mortality, including visceral obesity with insulin resistance, impaired glucose tolerance, atherosclerosis, systemic hypertension, dyslipidemia and hypercoagulability [3].

An increase in visceral fat is a phenotypic feature of CS. Importantly, our understanding of the mechanisms whereby increased central adiposity leads to metabolic alterations and cardiovascular morbidity has been largely based on the demonstration that adipose tissue secretes a number of cytokines and bioactive compounds, the so-called 'adipokines'. Adipokine, which include a variety of pro-inflammatory peptides, are involved in many physiological or pathological processes, including inflammation, endothelial damage, atherosclerosis, impaired insulin signaling, hypertension and bone remodeling. Adipokine dysregulation is a strong determinant of the 'low-grade' inflammatory state of obesity, promoting a cascade of metabolic aberrations leading to cardiovascular complications [4]. In CS, increased visceral adiposity [5, 6] is associated with altered production of adipokines, which contributes to the pathogenesis of the comorbidities observed in this condition. This review will highlight recent insights into the relationship between adipokines and cortisol, with particular regard to cardiovascular risk in CS. Because we will only focus on those adipokines that have been studied in CS, it is possible that other molecules that have been significantly associated with cardiovascular risk in other models of disease will not be discussed comprehensively in this review.

Cushing's Syndrome and Fat Distribution

Conflicting data have been reported regarding fat distribution in patients with CD compared to BMI-matched controls. For example, one study reported that overweight women with CD had higher percent trunk fat (as assessed by DXA) than BMI-matched controls [7], whereas another reported that total and trunk fat in obese women with CD were comparable to BMI-matched controls [5]. Although total and trunk fat in obese CD females did not differ from BMI-matched controls in the latter study, a strong correlation was observed in CD patients between absolute trunk fat and intra-abdominal visceral area as assessed by CT. Indeed, the abdominal visceral to subcutaneous fat ratio (V/S) was significantly greater in CD women than in either obese or normal-weight controls [5]. Interestingly, the increase in the V/S ratio does not differ between female and male patients with CS, suggesting that hypercortisolism may override gender-related differences in fat distribution [6].

It is important to emphasize that concomitant growth hormone deficiency (GHD) in states of cortisol excess (CS-GHD), both functional or based on tumor-related somatotrope dysfunction, is a common feature of active [8, 9] as well as cured CD [10]. Magiakou et al. [8] found a marked reduction of both spontaneous and stimulated GH secretion in children with CS, which persisted a year after surgical remission of hypercortisolism. In a small study on 16 adult patients with CS, GHD was reported in the entire group prior to surgery, with 69% of patients having severe GHD [9]. Pecori Giraldi et al. [10] showed that 65% of CS patients assessed within a median of 3.3 years of follow-up after surgical resolution of hypercortisolism had impaired GH secretion. Of note, the duration of hypercortisolism predicted postsurgical GHD, suggesting a direct, detrimental effect of glucocorticoid excess on GH secretion [10]. This effect may be related to alterations of either GHRH [11] or somatostatin release [12] by high levels of glucocorticoids, or a direct inhibition of pituitary somatotropes by glucocorticoids [13].

CS-GHD contributes to the metabolic alterations typically observed in this pathological condition, even many years after resolution of hypercortisolism [14], although the degree of dysfunction varies between CS and GHD. In a small study that compared body composition in patients with either CS or GHD to normal controls, percent trunk fat was highest in CS and lowest in normal controls, with patients with GHD having trunk fat intermediate between patients with CS and controls, although the difference between groups was not statistically significant [15].

Patients with CS-GHD have an increased prevalence of diabetes mellitus, hypertension and cardiovascular diseases as compared with GHD patients with previous nonfunctioning pituitary adenomas [16, 17]. Of note, Johannsson et al. [18] demonstrated that among cured CD patients with concomitant GHD, there was a trend towards reduction in body fat during 2 years of GH replacement. At the end of the treatment period, within-group reduction in body fat approached significance. Höybye et al. [17] showed a significant improvement of lipid profile in CS-GHD patients after 3 years of GH replacement.

Leptin

Leptin and Cardiovascular Risk

Leptin, the ob gene product, is an anorexigenic hormone mainly secreted by adipocytes in proportion to body fat (specifically subcutaneous fat) content. Levels are typically elevated in obesity, which is considered a leptin-resistant state [19]. Hyperleptinemia in obese individuals has been widely recognized as an independent cardiovascular risk factor associated with hyperinsulinemia and insulin resistance [20]. Compelling evidence also suggests a pathogenetic role of high leptin in atherothrombosis and endothelial dysfunction [20]. Leptin can induce release of reactive oxygen species and monocyte recruitment, promoting atherogenesis in bovine endothelial cells [21]. O'Rourke et al. [22] showed that in the presence of elevated glucose levels, leptin stimulated in vitro macrophage cholesterol ester synthesis, thus contributing to foam cell formation. A positive relationship between plasma leptin and coronary artery calcifications was shown in 860 nondiabetic healthy subjects after adjusting for age, gender and established cardiovascular risk factors [23]. Moreover, leptin was found to induce expression of C-reactive protein (CRP), a well-known marker of cardiovascular risk, in human coronary artery endothelial cells [24]. Leptin correlated with CRP levels in a case-control study including 377 men who subsequently experienced a coronary event. In this group of patients, leptin remained a significant, independent predictor of a coronary event even after adjusting for CRP [25].

Leptin and the HPA Axis

In vitro Studies

Leptin may impact the HPA axis at multiple levels. Heiman et al. [26] reported that leptin causes a dose-dependent inhibition of CRH release, and inhibits both fasting- and stress-related increases in ACTH secretion in primary cultures of rat pituitary cells in vitro, with a dose-dependent inhibition of CRH release. In that study, leptin did not have a direct effect on pituitary ACTH secretion [26]. In contrast, in another study, a direct stimulatory effect of leptin on ACTH release was observed in superfused mouse pituitary slices [27]. These data may suggest species-specific effects of leptin at the level of the pituitary, although different experimental approaches and methodologies may have also contributed to these contradictory findings. Furthermore, leptin has been demonstrated to directly inhibit ACTH-mediated, but not basal cortisol secretion from human adrenal cells in vitro in a dose-dependent manner [28]. This inhibition peaked after 24 h of exposure to human physiological leptin concentrations, suggesting a potential long-term effect of this adipokine on adrenal steroidogenesis [28]. In contrast, a dose-dependent enhancement of 11β-hydroxysteroid dehydrogenase type 1 activity (11β-HSD1), which converts inactive cortisone to cortisol, was seen in primary cultures of ob/ob mouse hepatocytes after addition of leptin, suggesting that this adipokine may in fact increase intra-tissue cortisol levels [29].

In vivo Studies: Animal Models

Similar to in vitro data, in vivo animal data regarding the effect of leptin on CRH and ACTH production are conflicting, and may again relate to differences in species and/or experimental design. Studies suggest a differential effect of acute versus chronic leptin administration on the HPA axis. Leptin acutely enhances hypothalamic CRH biosynthesis in rats, whereas its chronic administration exerts an inhibitory effect in mice [30]. A stimulatory effect of leptin on CRH expression was observed in the paraventicular nucleus (PVN) of rats 2–6 h after either intracerebroventricular (icv) or intraperitoneal administration of the peptide, consistent with its anorexigenic action [31, 32]. However, leptin infusion for 7 days was found to downregulate CRH expression in the mouse PVN and to prevent the compensatory rise of CRH in adrenalectomized mice [33]. A shorter period of subcutaneous leptin infusion did not have any effect on CRH expression in either mice or rats [30].

Interestingly, the effect of leptin on CRH secretion in rodents appears to be influenced by circulating glucocorticoids. CRH concentrations in the PVN rose by about 50% within 1–3 h of the icv injection of leptin in adrenalectomized rats, but this was not observed in animals with intact adrenals [34]. At the level of the pituitary, another study demonstrated that prolonged systemic administration of leptin increased ACTH concentrations in both adrenalectomized female rats and intact animals [30]. In contrast to these studies, chronic administration of leptin reduced POMC expression in the anterior pituitary of obese mice [35]. In rats, contrasting effects of leptin administration on steroid hormone secretion have been documented, with some studies showing a marked stimulation, and others describing a lowering effect of leptin on plasma corticosterone levels, depending on the route and duration of administration [30].

Complete leptin deficiency in the ob/ob mice is associated with decreased omental 11β-HSD1 activity and hypercorticosteronemia, which is reversed by chronic leptin

replacement [36], suggesting that leptin may facilitate tissue cortisol synthesis. In contrast, mice selectively overexpressing omental 11β-HSD1 present with typical features of the metabolic syndrome, including visceral obesity and hyperleptinemia. Although a direct relationship between cortisol and leptin cannot be inferred from this study, the data suggest that increased intracellular concentrations of glucocorticoids in the omentum may contribute to increased leptin secretion in central obesity [37].

Human Studies

In vivo studies in humans overall support an inhibitory effect of leptin on the HPA axis with an inverse relationship reported between leptin pulsatility and that of ACTH and cortisol in healthy males [38]. Koutkia et al. [39] reported a synchronicity between leptin and cortisol pulse dynamics in healthy men, with changes in leptin preceding a reciprocal change in cortisol during an overnight fast.

Interestingly, studies also suggest that exogenous cortisol may impact leptin secretion. A 48-hour exposure to dexamethasone (1 μmol/l) was reported to enhance leptin release from omental adipose tissue in vitro from women (but not men) [40]. In humans, glucocorticoid effects on leptin levels may be dose-, body weight-, gender- and duration-dependent. Administration of pharmacological doses of dexamethasone to normal subjects has been shown to cause either an acute, sustained, dose-dependent rise in blood leptin concentrations [41–45], or no significant effect [46–48]. For example, acute dexamethasone administration (10 mg over 4 days) increased leptin concentrations in obese subjects, which was associated with baseline leptin and BMI regardless of gender, age, or insulin sensitivity [43]. Putignano et al. [48] found a significant increase in plasma leptin after lower doses of dexamethasone (1 mg, 0.035 mg/kg, 0.007 mg/kg, 0.015 mg/kg) only in obese women, but not in men or in normal-weight subjects of either gender.

In humans, there are gender differences in leptin levels. Circulating levels of leptin, normalized to total fat mass, are 2- to 3-fold higher in premenopausal women compared with men [49]. Leptin is secreted in large amounts by subcutaneous fat, and higher leptin levels in women are also consistent with greater subcutaneous fat in women compared to men [50]. In addition, sexual dimorphism in leptin levels is mediated by sex steroids. Testosterone is inversely related to leptin and inhibits leptin secretion, in contrast to a slight stimulatory effect exerted by estradiol [51].

Soluble Leptin Receptor

The soluble leptin receptor (sOB-R) derives from the proteolytic cleavage of leptin membrane receptors. Binding of the sOB-R to leptin determines levels of free leptin in serum [52]. Chan et al. [53] reported that leptin and sOB-R were inversely related, with the former being able to reciprocally regulate its binding protein in several acute conditions. Interestingly, these authors found a positive correlation between cortisol and sOB-R, which was also reported in anorexia nervosa [53, 54]. Finally, in a study examining secretory patterns of sOB-R and cortisol in healthy men, Gavrila et al. [55] found that profiles of both hormones were strongly related, such that the peak for both was reached at approximately the same time in the morning, and the nadir of sOB-R occurred about 2 h after that of cortisol, suggesting a possible interplay between these hormones.

Leptin and Cushing's Syndrome

Several reports have documented elevated leptin concentrations in CS patients compared with normal-weight and BMI-matched controls [56–58] (table 1). Masuzaki et al. [57] showed that circulating leptin levels in CS patients (BMI range: 16.6–30.3) were similar to those in obese controls (BMI range: 27.8–56) but significantly higher than in healthy normal-weight subjects (BMI range: 16–23). Importantly, when leptin levels were evaluated in relation to the percentage of body fat measured by DEXA, they were significantly elevated in CS as compared with the entire control group. Consistent with these studies, Weise et al. [59] found a positive correlation between BMI and leptin in patients with active CS. However, no study thus far has evaluated the relative contribution of specific fat depots, namely visceral versus subcutaneous fat, to leptin levels in CS. This distinction is crucial, because subcutaneous fat is known to produce 2–3 times more leptin than omental fat [50]. CD, however, is characterized by a higher proportion of visceral compared to subcutaneous fat, with the latter being comparable in CD obese women and BMI-matched controls [5]. Of note, Widjaja et al. [60] reported higher leptin levels in CS males as compared with BMI-matched controls, likely consequent to greater trunk fat in CS, whereas no difference was observed in women with CS compared with BMI-matched controls, likely because of a similar proportion of subcutaneous fat in both groups.

A study of leptin pulsatility in 7 women with CD revealed a more than 2-fold elevation in daily leptin production rates as compared with age-, gender- and BMI-matched controls, which was driven by higher pulse am-

plitude rather than frequency [61]. Of note, approximate entropy of leptin release and cross-approximate entropy of cortisol and leptin were superimposable to those measured in controls. These findings argue in favor of an unexpected synchrony between leptin and cortisol in CD, with a preservation of normal responsiveness of fat cells to cortisol, despite its excessive and dysregulated production in that disorder. This evidence supports a possible direct influence of hypercortisolemia on hyperleptinemia in CD, although the same group did not find any correlation between leptin and cortisol secretion rates [61]. Similarly, Weise et al. [59] demonstrated maintenance of a normal diurnal leptin profile in 18 CS patients but did not observe any correlation between leptin and cortisol at any time point. This apparent dissociation between cortisol and leptin in CS was confirmed by the finding that CRH administration before and 10 days after surgical resolution of hypercortisolism in 12 CD patients did not impact leptin concentrations in spite of the significant differences in cortisol and ACTH response between the pre- and postoperative phases [59]. The maintenance of hyperleptinemia despite a fall in cortisol levels after transsphenoidal surgery (TSS) was confirmed by other investigators [58] and suggests that factors other than cortisol hypersecretion may play a role in leptin overproduction in CD, such as a persistence of fat distribution abnormalities. In contrast to acute normalization of cortisol, which does not cause reductions in leptin levels, short-term fasting for a 36-hour period is associated with a reduction in levels of leptin and insulin in both CS and obese patients, although not to the extent seen in healthy controls [62].

Although leptin levels do not change acutely after successful TSS for CD, levels do decrease chronically, likely due to decreased BMI, fat mass, and insulin levels with persistent cortisol normalization. Widjaja et al. [60] reported a significant decrease in leptin levels about 2.5 years after TSS in 9 CS patients as cortisol, insulin, and BMI decreased into the normal range.

Kresk et al. [63] observed a significant decrease in the ratio of leptin to sOB-R, a marker of leptin bioavailability, 9 months after curative TSS in CD patients, similar to findings in obese patients following bariatric surgery [64]. This is likely a consequence of reductions in body weight, fat mass and insulin levels with cortisol normalization. Indeed, in the report by Widjaja et al. [60], insulin was the only predictor of leptin levels in active CS. This is in agreement with several studies showing a direct relationship between leptin and insulin in humans [65, 66], with the latter being an independent predictor of decreased leptin levels in obese subjects after significant weight loss [67].

In another study of patients with CS, a significant postoperative decrease in leptin levels after an average of 31 months was associated with reductions in cortisol and trunk fat, and a concomitant decrease in interleukin (IL)-1Ra, the molecule thought to antagonize leptin signaling in the hypothalamus [68]. This finding indicates that similar to obesity, CS may be a state of leptin resistance, which at least in part is mediated by an elevation of IL-1Ra levels during the active phase of the disease [68].

Concomitant GH deficiency may also play a role in the hyperleptinemia associated with active CS, given that GH directly inhibits ob gene expression in visceral fat, as shown in obese rats [69]. Indeed, in GHD patients without Cushing's, leptin levels were significantly higher than in BMI-matched controls, but decreased to comparable levels after a year of GH replacement in parallel with a reduction of body fat [70]. These studies, however, do not conclusively determine whether elevations in leptin in states of GHD and cortisol excess are a direct consequence of low GH and high cortisol, or whether hyperleptinemia is consequent to coincident fat accumulation.

Overall, although the pathogenesis of hyperleptinemia in CS is not clear, it is interesting to speculate that the increase in leptin is a compensatory mechanism to antagonize glucocorticoid excess such as through inhibition of CRH release. Additionally, hyperleptinemia may represent an attempt to reduce the stimulatory effect of cortisol on food intake through leptin-mediated inhibition of the powerful orexigenic peptide neuropeptide Y and stimulation of the anorexigenic POMC and cocaine- and amphetamine-regulated transcript (CART) neurons [71].

In conclusion, most studies showed elevated leptin levels in active CS. Decreases in leptin secretion after correction of hypercortisolism appear to be dependent on the evaluation time (i.e. short-term vs. long-term after remission) and changes in body fat. Hyperleptinemia in CS may be a compensatory mechanism which is made ineffective by concomitant glucocorticoid excess and, possibly, by an obesity-like resistance to its action.

Adiponectin

Adiponectin and Cardiovascular Risk

Adiponectin is the protein product of the apM1 gene, which is mainly produced by visceral fat and circulates in blood in different forms of varying molecular weight [72, 73]. Low levels of adiponectin are a common feature of

obesity and are associated with insulin resistance [74]. In normal subjects, acute insulin infusion during a hyperglycemic hyperinsulinemic clamp caused a significant decrease in high-molecular-weight adiponectin, but not in total or low-molecular-weight adiponectin [75]. The ratio of high-molecular-weight/total adiponectin is a stronger predictor of insulin resistance and metabolic syndrome than total adiponectin levels [76] and has been shown to be significantly lower in diabetic as compared with nondiabetic subjects [75].

The insulin-sensitizing action of adiponectin is mediated through mitogen-activated protein kinase in muscle and liver with consequent increase in free fatty acid oxidation and reduced hepatic glucose production [74]. In addition, adiponectin has important anti-atherogenic and anti-inflammatory activity. It inhibits adhesion of monocytes to endothelial cells and transformation of macrophages to foam cells in vitro [77]. Moreover, adiponectin increases nitric oxide production in human aortic endothelial cells, and low levels of the adipokine are associated with impaired endothelium-dependent vasodilatation in patients with diabetes as well as in healthy subjects [78]. Adiponectin is inversely related to CRP and the cytokines tumor necrosis factor-α (TNF-α) and IL-6; the latter two are powerful inhibitors of adiponectin expression and secretion in cultured human adipose cells. These data suggest that the role of adiponectin in cardiovascular risk may be partly mediated by interplay with these pro-inflammatory molecules [79].

In a large cohort of men without cardiovascular disease, subjects having total adiponectin levels in the highest quintile had a significantly decreased risk of myocardial infarction compared with those in the lowest after 6 years of follow-up [80].

Adiponectin and the HPA Axis

In vitro and Animal Studies

An association between adiponectin and the HPA axis has been reported in various in vitro and in vivo animal models, and will be summarized here. Incubation of murine 3T3-L1 adipocytes with dexamethasone reduces expression of the adiponectin gene, an effect that is completely reversed following dexamethasone withdrawal for 24 h [79]. Similarly, Makimura et al. [81] reported that in ob/ob mice, which are characterized by low adiponectin levels and insulin resistance, adrenalectomy normalized expression of adiponectin and improved insulin resistance. These data suggest that glucocorticoids are inhibitory to adiponectin secretion and support the hypothesis that restoration of adiponectin levels may mediate the improvement in insulin sensitivity observed in adrenalectomized ob/ob mice. Transgenic overexpression of 11β-HSD1 in murine adipose tissue led to elevated intra-adipose corticosterone concentrations associated with low adiponectin expression, visceral obesity and insulin resistance [82]. Similarly, adipocyte-specific inactivation of glucocorticoid action in a transgenic murine model overexpressing 11β-HSD type 2 (11β-HSD2) led to a favorable metabolic profile, including reduction of food intake, increased expression of adiponectin, and a decrease in central fat accumulation and insulin resistance [83], further confirming an inverse relationship between glucocorticoids and adiponectin.

Human Studies

The few human studies that have been published thus far examining the impact of short-term glucocorticoid administration on adiponectin levels in humans are small in size and contradictory. Two studies reported no effect of glucocorticoid administration on adiponectin levels despite a concomitant impairment of insulin sensitivity in either normal [84] or obese [85] subjects. In contrast, another study reported an increase in circulating adiponectin levels in healthy male athletes who were administered 60 mg of prednisolone daily for a week [86], while Fallo et al. [87] demonstrated a significant decrease in adiponectin 30 and 60 min after a 25-mg injection of hydrocortisone in 5 healthy volunteers compared with placebo. Although differences in study populations and variations in study protocols including dose and duration of glucocorticoid administration may account for some of these discrepancies, it is likely that a longer exposure to elevated glucocorticoid levels may be necessary to detect changes in adiponectin concentrations associated with significant metabolic alterations.

Interestingly, a 24-hour profile of circulating adiponectin and cortisol in 6 young men indicated substantial differences in secretory patterns of the two hormones, despite a similar timing of peak hormone secretion. In particular, adiponectin, but not cortisol, plateaued during the day and then reached its nadir 2 h after cortisol at night [55].

Adiponectin and Cushing's Syndrome

A few small studies have reported adiponectin levels in patients with CS (table 1). These studies were limited by small size and heterogeneous characteristics of the samples, as well as by the fact that only total adiponectin was measured. As previously mentioned, adiponectin circulates in a variety of molecular forms and the pre-

Table 1. Adipokine patterns in CD

Adipokines	Pattern in active CS patients vs. BMI-matched controls	Pattern in CS after correction of hypercortisolism	
		change of levels vs. baseline	postsurgery time
Leptin	Increased [56–58, 62] Increased only in men [60] Unchanged [63]	Unchanged [58] Decreased [60, 63, 68]	10 days 9–36 months
Adiponectin	Decreased in non-obese; no difference in obese CD vs. non-obese [87] Unchanged [63, 89, 90]	Unchanged [63, 89, 90]	9–132 months
Resistin	Increased in females [63]	Unchanged [63]	9 months
TNF-α	Unchanged [68, 129, 130] Increased sTNF-R1 [90]	Increased in hypoadrenal patients [129] Increased sTNF-R1 vs. BMI-matched controls [90]	10 days 132 ± 72 months
IL-6	Unchanged [129, 130]	Increased in hypoadrenal patients [129] Increased vs. BMI-matched controls [90]	10 days 132 ± 72 months
Angiotensinogen	Increased expression of Ang II receptor 1A [166]	Not known	
PAI-1	Increased [172] Increased although not significantly [173]	Decreased vs. controls [173]	9 months
Ghrelin	Decreased [89] Increased; similar to controls with lower BMI [192]	Increased [89, 191]	3–24 months

sTNF-R1 = Soluble TNF-α receptor.

dominant molecular forms in CS have not been reported. In particular, it could be important to measure high-molecular-weight adiponectin, which, as noted, has been shown to significantly predict insulin resistance and metabolic syndrome in patients with diabetes [76]. This may yield results that differ from those reported thus far in this condition. This would be particularly true if associations of cortisol and adiponectin were driven by associated changes in insulin resistance, as is likely.

In one study, Fallo et al. [87] reported no difference in adiponectin levels between 11 obese patients with CD and BMI-matched controls, suggesting that increased body fat rather than hypercortisolism may be the main determinant of adiponectin in obese CS. However, this study also reported no difference in adiponectin levels in obese versus nonobese CD, and lower adiponectin levels in nonobese CD compared with nonobese controls, suggesting that hypercortisolemia does impact adiponectin levels directly, independently of body weight. The well-known inverse relationship between insulin sensitivity and adiponectin was confirmed in both obese and nonobese CD [87]. However, a limitation of this study was that female and male patients were analyzed together, despite the well-known sexual dimorphism in adiponectin regulation, with men having lower levels than women subsequent to inhibitory effects of testosterone on adiponectin release [88].

Another study of 14 women with CD failed to show any difference in adiponectin levels during the active phase of the disease compared with BMI-matched controls, possibly because of comparable insulin sensitivity in the two groups [89]. In addition, adiponectin levels remained unchanged 10 months after successful TSS compared with baseline values, despite a significant decrease of BMI, insulin resistance and cortisol, arguing against the regulation of adiponectin by endogenous glucocorticoids in CD [89]. These observations were confirmed by Kresk et al. [63].

Although Barahona et al. [90] reported that adiponectin levels were significantly lower in active CS as well as in cured CS after 11 ± 6 years as compared with healthy controls, this difference was no longer significant when patients were stratified based on their estrogen status.

As previously mentioned, GHD may coexist in patients with CD, and this may be a confounder in some of the published studies, although data regarding adiponec-

tin levels in GHD are also somewhat conflicting. Some reports showed no difference in total adiponectin concentrations between GHD patients at baseline and healthy controls [44, 91], and others described significantly lower levels in patients with GHD [92]. Giavoli et al. [92] also observed a significant elevation of this adipokine in GHD patients compared with baseline values after a year of treatment with GH, possibly related to favorable changes in body composition. However, this finding was not confirmed by others [44, 91].

In conclusion, future larger studies including isoform measurements are needed in order to conclusively assess whether a potential dysregulation in adiponectin production may contribute to the high metabolic and cardiovascular risk profile in CS patients.

Resistin

Resistin and Cardiovascular Risk

Resistin is a dimeric protein produced by macrophages and adipocytes especially those in visceral fat, and its name derives from its role in inducing insulin resistance in mice [93]. It regulates insulin sensitivity in skeletal muscle and liver, and its expression in human macrophages is reduced after incubation with rosiglitazone [94].

Resistin is elevated in obesity and is positively associated with insulin resistance and the degree of glucose intolerance in both humans and animal models [95]. Resistin is considered a marker of inflammation and a contributor to atherogenesis. In vitro studies report that resistin dose dependently induces proliferation of smooth muscle cells [96], and rh-resistin enhances the release of endothelin-I, vascular cell adhesion molecule (VCAM), and intercellular adhesion molecule-1 (ICAM-1) from human endothelial cells [97]. Resistin predicts coronary artery calcification in the metabolic syndrome [98] and was independently associated with arterial stiffness in the Baltimore Longitudinal Study of Aging [99]. Recent evidence from the Framingham Offspring Study suggests that elevated levels of resistin are inversely related to left ventricular fractional shortening, a marker of left ventricular systolic function [100]. Of note, resistin was an independent predictor of heart failure in the same cohort [101]. Moreover, resistin has been demonstrated to be independently associated with increased risk of myocardial infarction and ischemic stroke in a large cohort of middle-aged subjects, and in the Women's Health Initiative Observational Study [102, 103].

Resistin and the HPA Axis

In vitro and Animal Studies

In vitro and animal studies have shown a positive relationship between glucocorticoids and resistin. Incubation of 3T3-L1 adipose cells with dexamethasone enhanced expression of the resistin gene, suggesting that glucocorticoid-induced insulin resistance may be at least partly mediated by resistin [104]. However, another study using the same cell line could not confirm these results [105]. In prepubertal mice, resistin gene expression increased following injection of dexamethasone into the pituitary and decreased following adrenalectomy [106]. Notably, resistin gene expression was found in rat adrenal glands [107].

Resistin and Cushing's Syndrome

In the only study published thus far evaluating resistin levels in CS, resistin was significantly higher in 10 CS female patients compared with age-, sex-, and BMI-matched controls (table 1), and was positively associated with BMI, but not with urinary free cortisol or insulin [63]. Nine months after surgery, there was no significant decrease in resistin concentrations, despite a significant reduction in total body fat and trunk fat [63]. Interestingly, Ermetici et al. [108] reported significantly higher resistin levels in patients with adrenal incidentalomas as compared with controls.

The in vitro action of GH on resistin levels is controversial, and its incubation with 3T3-L1 adipocytes caused either inhibition or no effect on resistin expression [105]. Similar to recovered CS patients [63], a year of GH replacement in GHD patients did not significantly change resistin levels [109].

In summary, effects of resistin in hypercortisolemic states and/or associated GH deficiency remain to be determined.

Tumor Necrosis-Factor-α

TNF-α and Cardiovascular Risk

TNF-α is a pro-inflammatory cytokine with important regulatory effects on lipid metabolism, adipocyte function and insulin signaling [110]. Its expression has been shown to correlate with percent body fat and insulin resistance in humans [111]. Moreover, TNF-α produced by periarteriolar fat in obese rats impairs endothelium-dependent vasodilatation through inhibition of insulin-mediated release of nitric oxide [112]. Thus, TNF-α may be an important link between insulin resistance and vas-

cular disease. In addition, TNF-α has been reported to upregulate release of ICAM-1 and VCAM-1 on the surface of endothelial cells, which facilitate leukocyte adhesion to vessel walls [113]. These data confirm a prominent role of this adipokine in the pathogenesis of atherosclerosis and endothelial damage. Indeed, elevated levels of TNF-α predicted cardiovascular events in a cohort of 2,225 healthy elderly subjects during an average follow-up of 3.6 years [114]. Levels of TNF-α at the 95th percentile of the control distribution were associated with a 3-fold increase in recurrent acute ischemia in a case-control study including 544 patients [115].

TNF-α and the HPA Axis

In vitro and Animal Studies

Cross-talk between TNF-α and glucocorticoids is of paramount importance in modulating the immune response. TNF-α may also affect the HPA axis; it causes an elevation of glucocorticoid concentrations in acute inflammation [116]. TNF-α not only activates the HPA axis and enhances glucocorticoid production but also modifies tissue sensitivity to the anti-inflammatory effects of glucocorticoids [117].

Incubation of explanted rat hypothalami with graded concentrations of TNF-α showed a dose-dependent increase in CRH secretion, which was prevented by the preadministration of glucocorticoids. Of note, an increase in ACTH secretion was only observed after exposure to the highest doses of the cytokine [118]. In contrast, incubation of normal rat anterior pituitary cells with TNF-α inhibited CRH-stimulated ACTH release in a dose-dependent manner, without affecting basal secretion [119].

Finally, exposure of adult human adrenal cells to TNF-α enhanced basal and ACTH-stimulated cortisol release [120]. Because TNF-α expression has been demonstrated in human adrenal glands [120], one may hypothesize that TNF-α impacts cortisol secretion in adrenal tissue through a paracrine mechanism.

TNF-α administration potently enhanced transcriptional activity of 11β-HSD1 causing an increase in intracellular concentrations of cortisol [37]. Interestingly, coadministration of dexamethasone and insulin further potentiated the effect of TNF-α on 11β-HSD1 [37]. Thus, in conditions characterized by upregulation of 11β-HSD1, such as obesity, the concentration and action of cortisol were locally amplified by several factors that include TNF-α and other adipokines, triggering a 'vicious circle' or a 'fast-forward' feedback whereby adipose tissue and liver are continuously subject to the deleterious effects of glucocorticoid excess [121].

Studies in vitro assessing regulatory effects of glucocorticoids on TNF-α secretion in adipocytes have reported conflicting results, likely as a consequence of different experimental models. Sewter et al. [122] did not show any significant change in TNF-α release from either human adipose tissue or isolated adipocytes after 20 h of incubation with cortisol. In contrast, chronic treatment with dexamethasone for 15 days inhibited TNF-α expression in immortalized human pre-adipocytes in line with the immunosuppressive action of glucocorticoids [123]. Consistent with these data, glucocorticoids have been shown to inhibit TNF-α production in several animal models [124], while adrenalectomy causes elevated TNF-α levels during a septic insult in rats [125].

Human Studies

Similar to in vitro findings, studies examining the relationship between glucocorticoids and TNF-α in humans have reported conflicting results. One in vivo study showed that short-term dexamethasone administration in healthy men did not change TNF-α levels, despite concomitant occurrence of some dexamethasone-related metabolic perturbations [126]. In contrast, another study in healthy volunteers reported a significant suppression of TNF-α production following administration of either pharmacological or physiological doses of hydrocortisone (80 or 20 mg, respectively) [127]. Similarly, TNF-α release was suppressed after a stress-induced rise in glucocorticoid levels and a decline in TNF-α levels was observed concomitantly with circadian, physiological variations in cortisol, confirming the existence of a strict relationship between the two molecules [127].

Interestingly, in obese adolescent girls, high cortisol levels were an independent and direct predictor of TNF-α receptor 2, consistent with obesity being a pro-inflammatory state sustained by a relative and chronic excess of cortisol [128].

TNF-α and Cushing's Syndrome

Three observational studies have reported that circulating TNF-α levels are within the normal range in CS patients [68, 129, 130] (table 1), and do not correlate with cortisol levels [68]. Another study assessing TNF-α concentrations in 7 CD patients reported that basal TNF-α levels were not significantly higher in the inferior petrosal sinus ipsilateral to the ACTH-secreting adenoma than in the contralateral sinus and peripheral blood [131]. Of note, administration of CRH induced a significant rise in ipsilateral TNF-α levels as compared with the other two sites, confirming in vitro evidence of an acute stimula-

tory effect of CRH on TNF-α secretion [131]. Indeed, incubation of cells from ACTH-secreting adenomas with CRH led to a significant increase in TNF-α levels in the medium as compared with pre-exposure concentrations [131]. Barahona et al. [90] reported that levels of the soluble TNF-α receptor 1 were significantly higher in patients with active CS than in controls.

These in vivo data intriguingly suggest that the physiological interplay between cortisol and TNF-α is altered in the presence of cortisol excess and that levels of the cytokine are not as low as expected. It has been speculated that TNF-α levels, although in the normal range, are inappropriately high in CS in the face of chronic hypercortisolism, contributing to the persistence of a low-grade inflammatory state. This phenomenon may be a consequence of a reduced sensitivity of TNF-α to the inhibitory action of cortisol and/or a concomitant enhancement of inflammation by other factors, such as visceral fat, other adipokines and GHD, which antagonize direct glucocorticoid effects [132]. This may partly explain the lack of correlation with cortisol levels observed in some studies [68, 130].

Of note, TNF-α levels were shown to increase early after TSS in patients with postoperative hypocortisolism [129]. Slightly lower TNF-α levels, though still higher than in the preoperative phase, were observed 10 days after TSS in patients on replacement doses of dexamethasone [129]. These findings support the hypothesis that short-term decreases in cortisol induce metabolic and adipokine changes, which, in turn, restore physiological regulation of TNF-α secretion. Yet, a state of low-grade inflammatory activation may persist after cure of CS. Barahona et al. [90] reported that soluble TNF-α receptor 1 was significantly higher both in patients with active CS and in patients who had been surgically cured of hypercortisolism for an average of 11 years compared with normal controls suggesting that TNF-α signaling may be persistently altered in CS patients even after long-term cure. This may maintain a 'low-grade inflammation state' in cured CS patients, leading to a persistently elevated cardiovascular risk profile [90]. Another possible explanation for persistent abnormalities may be glucocorticoid replacement, particularly if the dose is supraphysiologic. In the Barahona study [90], nearly one-third of patients were taking glucocorticoid replacement.

Of note, a recent paper evaluating adipokine levels in 20 patients with adrenal incidentaloma (of whom only 3 had subclinical CS) found higher TNF-α concentrations in these patients as compared with age-, sex-, and BMI-matched controls, suggesting that adrenal tissue may autonomously produce TNF-α [108].

GH overexpression in rats and mice is associated with reduced TNF-α levels [110]. However, data are conflicting and TNF-α has recently been shown to be higher in patients with acromegaly as compared with normal controls [133]. A study in humans with GHD showed a 220% increase in concentrations of this adipokine and a subsequent significant reduction after starting GH replacement [134]. The impact of associated GHD in some patients with CS on TNF-α levels is unclear.

In conclusion, the physiological relationship between glucocorticoids and TNF-α may be blunted in CS, thus contributing to a state of chronic inflammation, which may persist long-term after remission of glucocorticoid excess.

Interleukin-6

IL-6 and Cardiovascular Risk

IL-6 is a cytokine which circulates as a glycosylated protein and has a wide range of actions including promotion of coagulation and immune/inflammatory reaction [135]. IL-6 is secreted by several cell types, including fibroblasts, endothelial cells and adipocytes, and plasma levels are significantly upregulated in human obesity and insulin resistance [136]. IL-6 administration directly altered insulin signaling in murine hepatocytes and 3T3-L1 adipocytes, and affected insulin-induced glycogenesis in liver cells [137]. A direct relationship has been observed between IL-6 in fatty tissue and insulin resistance in human obesity [138], and IL-6 has also been proven to be an important link between inflammation and atherosclerosis [139]. Indeed, IL-6 is induced by TNF-α in vascular tissue and has been shown to regulate CRP production in smooth muscle cells, which negatively affects expression of adhesion molecules, and endothelial function [139]. Elevated levels of IL-6 were associated with a doubling of the risk of cardiovascular and all-cause mortality in a cohort of 1,293 healthy, elderly subjects followed prospectively for a mean of 4.6 years [140], and had an important prognostic value in patients with unstable angina [141].

IL-6 and the HPA Axis

In vitro and Animal Studies

Similarly to other cytokines, IL-6 directly stimulates the HPA response to stress and inflammation. Incubation of rat hypothalami with IL-6 induced a rapid and dose-dependent release of CRH, and this effect was also observed in vivo after icv injection of IL-6 [142]. However, in vitro studies evaluating the effects of IL-6 on

ACTH synthesis and secretion have demonstrated contradictory findings, with a direct stimulatory effect on corticotrope cells [142], yet inhibitory effects on CRH-mediated ACTH secretion in rat anterior pituitary cell cultures [132]. Interestingly, activation of the immune response in mice lacking both CRH and IL-6 led to the release of significantly lower corticosterone levels than those measured in animals deficient in CRH or IL-6 alone, suggesting that IL-6 is an important CRH-independent stimulator of the HPA axis [143].

Indeed, IL-6 is able to directly and dose dependently induce the release of corticosteroids by animal and human cells in vitro and in vivo, and similar to TNF-α is considered an intra-adrenal factor regulating adrenal steroidogenesis [119]. Of note, it has been demonstrated that IL-6 and ACTH may act synergistically to enhance the production of corticosterone in rat adrenal cells [144]. Thus, it is possible that stress-induced increases in IL-6 contribute to the maintenance of homeostasis through the concomitant rise in glucocorticoid secretion.

Incubation of corticotrope adenoma cells with dexamethasone significantly inhibited intratumoral IL-6 release [145]. Adrenalectomized rats have elevated IL-6 levels, which significantly decrease after administration of corticosterone [146]. Although the dose-dependent inhibition of IL-6 by glucocorticoids has been documented in many studies in animal and human cell cultures, including adipocytes [147], some studies suggest that suppression is partial, likely from concomitant effects of other factors, such as catecholamines [132].

Human Studies

Human studies have suggested that the interaction between glucocorticoids and IL-6 is modulated by a complex network of signals, including TNF-α.

Administration of 80 mg of hydrocortisone to healthy volunteers suppressed IL-6 release, whereas stress-induced levels of glucocorticoids had no effect [127]. This observation, along with the finding that circadian variations of cortisol were not associated with decreased IL-6 secretion [127], seems to confirm that the relationship between glucocorticoids and IL-6 in humans is at least in part mediated by other factors, including TNF-α and/or catecholamines. Interestingly, Papanicolau et al. [148] showed that a high-intensity exercise test in healthy men caused a peak of catecholamines which was positively associated with a concurrent peak of IL-6. Pretreatment with dexamethasone or hydrocortisone attenuated, but did not block, the IL-6 peak.

It is well known that the stimulatory action of IL-6 on the HPA axis represents the basis for the immunomodulatory treatment of many illnesses including tumors. Daily administration of IL-6 to patients with cancer causes a significant rise in ACTH and cortisol levels over 7 days [149].

IL-6 and Cushing's Syndrome

Data regarding IL-6 levels in CS are contradictory (table 1). One study reported that IL-6 levels are not suppressed in patients with active CS [129] and do not differ significantly from age-, sex- and BMI-matched controls [130]. However, Papanicolau et al. [129] showed that in patients with active CS, IL-6 may be more responsive to the tonic inhibitory action of glucocorticoids than other cytokines; IL-6 levels rose dramatically in the immediate postoperative period, when patients became hypoadrenal. This increase was more consistent than that observed for other adipokines, and was partially reversed by subsequent glucocorticoid replacement [129]. These data suggest the existence of a tonic negative feedback loop between endogenous cortisol and IL-6. However, the data are by no means conclusive, and another study [90] showed that IL-6 concentrations were significantly higher in women with active CS than in gender-, age-, and BMI-matched controls, and remained as elevated in cured patients even after a mean of 11 years of cure [90].

In 20 patients with cortical adenomas, of whom only 5 had been diagnosed with subclinical hypercortisolism, IL-6 levels were elevated compared with controls and were inversely related to urinary free cortisol concentrations [108]. In addition, Kushlinskii et al. [150] found that circulating IL-6 levels in patients with adrenal tumors were significantly higher than those in healthy controls, and were highest in patients with adrenocortical tumors. These data suggest possible autonomous production of this adipokine by adrenal tissue.

As previously mentioned, patients with CD often have GHD and this may affect IL-6 production. Although in vitro studies showed that GH administration is able to induce IL-6 expression in 3T3-L1 adipocytes, in vivo observations have documented an inverse relationship between GH and IL-6. In particular, Serri et al. [134] found a 340% increase in IL-6 in GHD patients as compared with controls, and a decrease in IL-6 following GH replacement.

In conclusion, inappropriately high levels of IL-6 in Cushing's (which may or may not be related to GHD) represent another important pathogenic component of the inflammatory-related vascular and metabolic complications associated with chronic glucocorticoid excess.

Angiotensinogen

Angiotensinogen and Cardiovascular Risk

Angiotensinogen (AT), the precursor of the vasoactive peptide angiotensin II, is predominantly produced by the liver, followed by adipose tissue. AT is higher in obese than lean subjects, and is positively associated with blood pressure [151]. Indeed, AT-mRNA expression in adipose tissue, and specifically in omental fat, is upregulated in obese patients [152]. In addition, AT overexpression in murine adipose tissue leads to adipocyte hypertrophy, an increase in fat mass, increased plasma AT levels, hyperinsulinemia, hyperleptinemia, and hypertension, whereas the opposite metabolic pattern is observed in the AT knock-out model [153]. AT re-expression in AT-null mice, which are lean and hypotensive, restores normal blood pressure [154].

Angiotensinogen and the HPA Axis

Dexamethasone administration induces AT gene expression and secretion in cultured mature mouse adipose cells and explants of rat adipose tissue ex vivo [155]. In addition, an upregulation of AT has been described in transgenic mice overexpressing adipose 11β-HSD-1 [156]. It would be intriguing to speculate that glucocorticoids may stimulate AT secretion, which, in turn, may act as either a vasoconstrictor or trophic factor for adipose tissue through its activation products (e.g. angiotensin II). Indeed, angiotensin II promotes differentiation of preadipocytes to adipocytes through release of prostacyclin [157] and cortisol has been shown to increase angiotensin II type 1 receptor gene expression in human adipocytes in a time- and dose-dependent manner [158]. However, data published thus far do not indicate a direct, causal relationship between AT and cortisol in human models [159]. Data regarding acute effects of AT or angiotensin infusion on HPA axis function are limited, with some studies describing no effect [160, 161], while others report a decrease in ACTH or cortisol secretion [162, 163] in humans. Of note, a recent paper by Sanchez-Lemus et al. [164] showed that angiotensin II receptor 1A (AT1A) blockade increased basal circulating levels of corticosterone in rats.

Angiotensinogen and Cushing's Syndrome

Whether AT may play a role in any of the clinical features of chronic glucocorticoid excess is unclear because the scarce data published in CS so far are inconclusive. Of interest, one study showed that hypertension in CS is largely mediated by angiotensin II [165].

Shibata et al. [166] found an increase in the expression of AT1A in both mononuclear leukocytes and platelets of 7 patients with unilateral adrenal cortical adenomas causing CS compared with controls, which was reversed after adrenalectomy [166]. AT1A gene expression in these adrenal tumors was decreased compared with that from adrenal glands of control subjects (table 1).

Plasminogen Activating Inhibitor-1

Plasminogen Activating Inhibitor-1 and Cardiovascular Risk

Plasminogen activating inhibitor (PAI-1), which is produced by liver and adipose tissue, inhibits the activity of tissue-type plasminogen activator, an anticlotting factor, thereby favoring thrombus formation over ruptured atherosclerotic plaques. PAI-1 expression is elevated in visceral obesity, insulin resistance and hypertriglyceridemia, and its levels appear to predict risk for future development of both type 2 diabetes and cardiovascular disease [167, 168].

PAI-1 and the HPA Axis

Dexamethasone has been observed to induce a time- and dose-dependent expression and release of PAI-1 from subcutaneous and especially omental fat in vitro [169]. Interestingly, this stimulatory effect of glucocorticoids on PAI-1 production by adipose tissue appeared to be inhibited by coincubation with an inhibitor of 11β-HSD-1 [170]. Indeed, in situ hybridization revealed increased PAI-1 mRNA expression in visceral adipose tissue of obese subjects, which was positively related to the expression of 11β-HSD-1 mRNA, suggesting that local conversion of cortisone to cortisol may contribute to the observed elevation of PAI-1 levels in obesity [170]. Similarly, a 3-hour intravenous infusion of hydrocortisone enhanced PAI-1 circulating concentrations in both obese and normal women, and the percent increase between 180 and 240 min was significantly higher in obese subjects compared with controls [171].

PAI-1 and Cushing's Syndrome

CS has been shown to be associated with increased levels of PAI-1 compared with healthy controls, which may relate to the hypercoagulable state frequently observed in patients with this condition [172, 173] (table 1).

Of note, PAI-1 positively correlates with BMI in active disease. Levels are comparable to controls after a median of 36 months from surgical remission of hypercortisolism [173].

Similarly, GHD patients have increased levels of PAI-1, and this pattern correlates with BMI and waist-to-hip ratio [174, 175]. Indeed, GH was shown to enhance PAI-1 expression and secretion from adipocytes in vitro, although it has also been hypothesized that GH may indirectly downregulate PAI-1 production through its modulatory effect on the nitric oxide release from endothelial cells [175]. GHD may represent another cause of high PAI-1 levels in CS.

Ghrelin

Ghrelin and Cardiovascular Risk

Ghrelin is a 28-amino-acid acylated peptide mainly secreted by the stomach and represents the principal endogenous ligand for growth hormone secretagogue receptor (GHS-R) type 1a, whose expression is observed in the hypothalamo-pituitary region [176]. Ghrelin is also synthesized by the hypothalamus, pituitary, pancreas, kidney, heart, thyroid and Leydig cells. Although ghrelin does not meet the definition of an adipokine, it is involved in the regulation of glucose metabolism and lipogenesis both directly and through interactions with adipokines and is important to consider. Increasing evidence indicates that ghrelin, in addition to its GH secretagogue and orexigenic effects, also impacts diverse processes including ACTH and PRL secretion, glucose and lipid metabolism, gastric motility and acid secretion, cardiac function, sleep, and reproduction. In addition, ghrelin shows antiproliferative effects both in vivo and in vitro [176]. Ghrelin is present in the blood in two forms: the desacylated and the acylated form. The latter, which circulates at 2.5-fold lower concentrations than the unacylated, binds to the GHS receptors and mediates most of the endocrine actions of the molecule [177].

Ghrelin is known to stimulate the differentiation of pre-adipocytes into adipocytes and antagonize lipolysis [176]. Ghrelin levels are inversely correlated with BMI and insulin resistance, and food has an inhibitory effect on ghrelin, which is attenuated in obesity [176].

This orexigenic peptide also exerts anti-inflammatory and cardioprotective actions, partly mediated through its inhibitory actions on TNF-α, IL-1β, and IL-6 [178]. Ghrelin has been shown to inhibit mononuclear cell binding and nuclear factor-κB activation in human endothelial cells in vitro and in a rat model in vivo [179]. In addition, it has been found to directly increase endothelial nitric oxide synthase expression in humans with metabolic syndrome, improving endothelial dysfunction [180]. Moreover, ghrelin infusion increases cardiac output and reduces systemic vascular resistance in healthy humans [181, 182].

Ghrelin and the HPA Axis

In vitro and Animal Studies

Ghrelin acutely induces ACTH secretion, an effect that decreases following chronic administration of ghrelin [183]. The ghrelin-induced ACTH release is mediated by central mechanisms involving CRH and neuropeptide Y [176]. Ghrelin released from the rat hypothalamus stimulates CRH production [184]. In addition, the ability of GH secretagogues (GHS) to stimulate ACTH and cortisol requires an intact hypothalamic-pituitary axis as this effect is mediated by CRH rather than a direct effect of GHS on pituitary corticotropes [185]. In normal subjects, the extent of ACTH response to GHS does not differ from that following CRH administration and is sensitive to negative feedback inhibition by cortisol [186].

However, some direct stimulatory action of ghrelin on the pituitary cannot be ruled out, as GHS receptors (GHS-R) have been found on normal pituitary cells as well as corticotrope adenomas as well as normal pituitary cells [187], and a direct stimulatory effect of ghrelin on ACTH release in such tumors has been demonstrated in vitro [188]. Of note, even ectopic ACTH-secreting tumors show an exaggerated response to GHS, arguing against the use of ghrelin in differentiating between pituitary and extrapituitary causes of ACTH-dependent hypercortisolism [176].

Importantly, both the ghrelin receptor (GHS-R1a) and ghrelin mRNA have been detected in normal adrenal glands as well as in cortisol-secreting adenomas, suggesting that ghrelin may have an additional pathophysiological role in the regulation of the HPA axis [189].

Glucocorticoids may in turn affect ghrelin secretion. Although short-term dexamethasone treatment in rats did not cause a significant change in pituitary ghrelin expression, 1 week of treatment led to a significant decrease in ghrelin expression compared with controls [190]. Similarly, daily administration of 30 mg of prednisolone for 5 days to 8 healthy males induced a significant decrease in ghrelin levels [191]. It is possible that some of these changes in ghrelin levels are mediated by changes in insulin.

Ghrelin and Cushing's Syndrome

Data available on ghrelin in CS do not conclusively define its relative contribution to the metabolic and cardiovascular features associated with chronic hypercorti-

solism. Ghrelin levels have been reported as both increased and decreased in CS [89, 191, 192]. A potential limitation to the studies published so far is that only total ghrelin was measured and the acylated form of the molecule is responsible for the neuroendocrine actions of ghrelin, including its regulation of the HPA axis. Importantly, desacylated ghrelin appears to have an opposite effect on glycometabolic status compared with the acylated form, and has been reported to induce the growth of adrenocortical tumors [177].

In patients with CD, the ACTH response to ghrelin is exaggerated and even higher than that observed following CRH administration in the same subjects [176].

Otto et al. [191] reported that a single fasting plasma ghrelin measurement in 5 CS patients was significantly higher 24 months after successful surgical correction of hypercortisolism compared with values before intervention. Of note, this increase in ghrelin levels was inversely related to the significant postsurgical decrease in BMI, suggesting that the change in ghrelin may be mediated by the progressive weight loss induced by remission of CS, and the associated reduction in insulin levels (table 1). Similar findings were reported by Libè et al. [89], who noted that higher fasting ghrelin levels in 14 CD patients 10 months after surgical resolution of hypercortisolism compared with baseline were associated with a significant reduction in BMI and insulin levels over this period. Because insulin is known to inhibit ghrelin secretion [193], it is possible that insulin resistance associated with CS is responsible for lower ghrelin levels during the active stage of the disease. However, Libè et al. [89] could not find any correlation between presurgical ghrelin and insulin levels.

In a study by Giordano et al. [192], ghrelin levels measured every 15 min for 3 h in 8 CD patients were comparable to those found in normal controls with significantly lower BMI, despite significantly higher insulin and glucose levels in CD. Although the well-known inhibitory effect of glucose and insulin on ghrelin levels appears to be blunted in CD, acute administration of ghrelin to CD patients causes the expected physiological increase in glucose, suggesting that in the presence of chronic hypercortisolism, some metabolic actions of ghrelin are preserved. Of interest, no correlation was found between ghrelin and cortisol levels either before [89, 192] or a few days after successful surgery [89]. However, the sample size was small in these studies.

Published data do not show an association between GH and ghrelin levels in CS, although ghrelin is a GHS and CS is often associated with GHD [194, 195]. Interestingly, a recent study found that ghrelin suppression during insulin-induced hypoglycemia was similar in patients with GHD and normal subjects, and total ghrelin levels were not associated with either GH or cortisol levels [196]. In contrast, another study reported lower levels of total ghrelin in GHD patients at baseline compared with controls, followed by a subsequent increase in ghrelin levels after a year of GH treatment, which was associated with a significant decrease in body fat as compared with controls [92].

In conclusion, low ghrelin levels may contribute to increased cardiovascular risk in CD similar to that observed in obesity, but future studies are needed to further clarify this point, particularly by differentiating the interactions of acylated versus desacylated ghrelin with the HPA axis.

Conclusions

Adipokines are at the center of a complex network of signals that regulate metabolism and cardiovascular function. Imbalance of adipokine production is associated with increased cardiovascular risk in several conditions characterized by central fat accumulation, including CS. Indeed, elevation of leptin and resistin levels as well as the release of pro-inflammatory cytokines, such as TNF-α and IL-6, may lead to the high cardiovascular morbidity observed during the active phase of CS. Likewise, persistent impairment of adipokine secretion may contribute to the increased long-term cardiovascular risk in patients cured of CS described in some earlier reports. Future studies are needed to fully elucidate the interactions between clinical or subclinical increases in cortisol production and dysregulated adipokine secretion in CS, providing new insights into the pathogenesis of complications associated with this potentially lethal disease.

References

1 Newell-Price J, Trainer P, Besser M, Grossman A: The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr Rev 1998;19:647–672.

2 Extabe J, Vazquez JA: Morbidity and mortality in Cushing's disease: an epidemiological approach. Clin Endocrinol 1994;40:479–484.

3 Pivonello R, Faggiano A, Lombardi G, Colao A: The metabolic syndrome and cardiovascular risk in Cushing's syndrome. Endocrinol Metab Clin North Am 2005;34:327–339.

4 Halberg N, Wernstedt-Asterholm I, Scherer PE: The adipocyte as an endocrine cell. Endocrinol Metab Clin North Am 2008;37:753–768.
5 Wajchenberg BL, Bosco A, Martins Marone M, Levin R, Rocha M, Lerario AC: Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. J Clin Endocrinol Metab 1995;80:2791–2794.
6 Rockall AG, Sohaib SA, Evans D, Kaltsas G, Isidori AM, Monson JP, Besser GM, Grossman AB, Reznek RH: Computed tomography assessment of fat distribution in male and female patients with Cushing's syndrome. Eur J Endocrinol 2003;149:561–567.
7 Garrapa GGM, Pantanetti P, Arnaldi G, Mantero F, Faloia E: Body composition and metabolic features in women with adrenal incidentaloma or Cushing's disease. J Clin Endocrinol Metab 2001;86:5301–5306.
8 Magiakou MA, Mastorakos G, Gomez MT, Rose SR, Chrousos GP: Suppressed spontaneous and stimulated growth hormone secretion in patients with Cushing's disease before and after surgical cure. J Clin Endocrinol Metab 1994;78:131–137.
9 Tzanela M, Karavitaki N, Stylianidou C, Tsagarakis S, Thalassinos N: Assessment of GH reserve before and after successful treatment of adult patients with Cushing's syndrome. Clin Endocrinol 2004;60:309–314.
10 Pecori Giraldi F, Andrioli M, De Marinis L, Bianchi A, Giampietro A, De Martin M, Sacco E, Scacchi M, Pontecorvi A, Cavagnini F: Significant GH deficiency after long-term cure by surgery in adult patients with Cushing's disease. Eur J Endocrinol 2007;156:233–239.
11 Senaris RM, Lago F, Coya R, Pineda J, Dieguez C: Regulation of hypothalamic somatostatin, growth-hormone releasing hormone, and growth hormone receptor messenger ribonucleic acid by glucocorticoids. Endocrinology 1996;137:5236–5241.
12 Wehrenberg WB, Bergman PJ, Staag L, Ndon G, Giustina A: Glucocorticoid inhibition of growth hormone in rats: partial reversal with somatostatin antibodies. Endocrinology 1990;127:2705–2708.
13 Leal-Cerro A, Soto A, Martinez MA, Alvarez P, Isidro L, Casanueva F, Dieguez C, Cordido F: Effect of withdrawal of somatostatin plus growth hormone (GH)-releasing hormone is a stimulus of GH secretion in Cushing's syndrome. Clin Endocrinol 2002;57:745–749.
14 Colao A, Pivonello R, Spiezia A, Faggiano A, Ferone D, Filippella M, Marzullo P, Cerbone G, Siciliani M, Lombardi G: Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. J Clin Endocrinol Metab 1999;84:2664–2672.
15 Burt MG, Gibney J, Ho KKY: Characterization of the metabolic phenotypes of Cushing's syndrome and growth hormone deficiency: a study of body composition and energy metabolism. Clin Endocrinol 2006;64:436–443.
16 Webb SM, Mo D, Lamberts SWJ, Melmed S, Cavagnini F, Pecori Giraldi F, Strasburger CJ, Zimmermann AG, Woodmansee WW, on behalf of the International HypoCCS Advisory Board: Metabolic, cardiovascular, and cerebrovascular outcomes in growth-hormone deficient subjects with previous Cushing's disease or non-functioning pituitary adenoma. J Clin Endocrinol Metab 2010;95:630–638.
17 Höybye C, Ragnarsson O, Jönsson PJ, Koltowska-Häggström P, Trainer P, Feldt-Rasmussen U, Biller BM: Clinical features of GH deficiency and effects of 3 years of GH replacement in adults with controlled Cushing's disease. Eur J Endocrinol 2010;162:677–684.
18 Johannsson G, Stibrant Sunnerhagen K, Svensson J: Baseline characteristics and the effects of two years of growth hormone replacement therapy in adults with growth hormone deficiency previously treated for Cushing's disease. Clin Endocrinol 2004;60:550–559.
19 Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF: Serum immunoreactive leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292–295.
20 Martin SS, Qasim A, Reilly MP: Leptin resistance: a possible interface of inflammation and metabolism in obesity-related cardiovascular disease. J Am Coll Cardiol 2008;52:1201–1210.
21 Yamagishi SI, Edelstein D, Du XL, Kaneda Y, Guzmán M, Brownlee M: Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase A. J Biol Chem 2003;276:25096–25100.
22 O'Rourke L, Gronning LM, Yeman SJ, Shepherd PR: Glucose-dependent regulation of cholesterol ester metabolism in macrophages by insulin and leptin. J Biol Chem 2002;277:42557–42562.
23 Qasim A, Mehta N, Tadesse MG, Wolfe ML, Rhodes T, Girman G, Reilly MP: Adipokines, insulin resistance and coronary artery calcification. J Am Coll Cardiol 2008;52:231–236.
24 Singh P, Hoffmann M, Wolk R, Shamsuzzaman AS, Somers VK: Leptin induces C-reactive protein expression in vascular endothelial cells. Arterioscler Thromb Vasc Biol 2008;27:e302–e307.
25 Wallace AM, Mc Mahon AD, Pacjard CJ, Kelly A, Shepherd J, Graw A, Sattar N: Plasma leptin and the risk of cardiovascular disease in the West of Scotland Coronary Prevention Study (WOSCOPS). Circulation 2001;104:3052–3056.
26 Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS: Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. Endocrinology 1997;138:3859–3863.
27 Raber J, Chen S, Mucke L, Feng L: Corticotropin-releasing factor and adrenocorticotrophic hormone as potential central mediators of OB effects. J Biol Chem 1997;272:15057–15060.
28 Pralong FP, Roduit R, Waeber G, Castillo E, Mosimann F, Thorens B, Gaillard RC: Leptin inhibits directly glucocorticoid secretion by normal human and rat adrenal gland. Endocrinology 1998;139:4264–4268.
29 Liu Y, Nakagawa Y, Wang Y, Li R, Ohzeki T, Friedman TC: Leptin activation of corticosterone production in hepatocytes may contribute to the reversal of obesity and hyperglycemia in leptin deficient ob/ob mice. Diabetes 2003;52:1409–1416.
30 Malendowicz L, Rucinski M, Belloni A, Ziolkowska A, Nussedorfer G: Leptin and the regulation of the hypothalamic-pituitary-adrenal axis. Int Rev Cytol 2007;263:63–102.
31 Uehara Y, Shimizu H, Ohtani K, Sato N, Mori M: Hypothalamic corticotropin-releasing hormone is a mediator of anorexigenic effect of leptin. Diabetes 1998;47:890–893.
32 Nishiyama M, Makino S, Asaba K, Hashimoto K: Leptin effects on the expression of type-2 CRH receptor mRNA in the ventromedial hypothalamus in the rat. J Neuroendocrinol 1999;11:307–314.
33 Arvaniti K, Huang Q, Richard D: Effects of leptin and corticosterone on the expression of corticotrophin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. Neuroendocrinology 2007;73:227–236.
34 Jang M, Mistry A, Swick AG, Romson DR: Leptin rapidly inhibits hypothalamic neuropeptide Y secretion and stimulates corticotrophin-releasing hormone secretion in adrenalectomized mice. J Nutr 2007;130:2813–2820.
35 Renz M, Tomlison E, Hultgren B, Levin N, Gu QM, Shimkets RA, Lewin DA, Stewart TA: Quantitative expression analysis of genes regulated by both obesity and leptin reveals a regulatory loop between leptin and pituitary-derived ACTH. J Biol Chem 2000;275:10429–10436.
36 Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, MacKellar W, Rosteck PR, Schoner B, Smith D, Tinsley FC, Zhang XY, Heiman M: The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 1995;377:530–532.
37 Iwasaki T, Takayasu S, Nishiyama M, Tsugita M, Taguchi T, Asai M, Yoshida M, Kambayashi M, Hashimoto M: Is the metabolic syndrome an intracellular Cushing state? Effects of multiple humoral factors on the transcriptional activity of the hepatic glucocorticoid-activating enzyme (11β-hydroxysteroid dehydrogenase type 1) gene. Mol Cell Endocrinol 2008;285:10–18.

38 Licinio J, Mantzoros C, Negrao CB, Cizza G, Wong ML, Bongiorno PB, Chrousos GP, Karp B, Allen C, Flier JS, Gold PW: Human leptin levels are pulsatile and inversely related to pituitary-adrenal function. Nat Med 1997;3:575–579.

39 Koutkia P, Canavan B, Johnson ML, De Paoli A, Grinspoon S: Characterization of leptin pulse dynamics and relationship to fat mass, growth hormone, cortisol, and insulin. Am J Physiol Endocrinol Metab 2003;285:E372–E379.

40 Casabiell X, Pineiro V, Peino R, Lage M, Camina J, Gallego R, Vallejo LG, Dieguez C, Casanueva FF: Gender differences in both spontaneous and stimulated leptin secretion by human omental adipose tissue in vitro: dexamethasone and estradiol stimulate leptin release in women, but not in men. J Clin Endocrinol Metab 1998;83:2149–2155.

41 Miell JP, Englaro P, Blum WF: Dexamethasone induces an acute and sustained rise in circulating leptin levels in normal human subjects. Horm Metab Res 1996;28:704–707.

42 Newcomer JW, Selke G, Melson AK, Gross J, Vogler GP, Dagogo-Jack S: Dose-dependent cortisol-induced increases in plasma leptin concentration in healthy humans. Arch Gen Psychiatry 1998;55:995–1000.

43 Dagogo-Jack S, Selke G, Melson AK, Newcomer JW: Robust leptin secretory responses to dexamethasone in obese subjects. J Clin Endocrinol Metab 1997;82:3230–3233.

44 Janssen JA, Huizenga NA, Stolk RP, Grobbee DE, Pols HA, de Jong FH, Attanasio AM, Blum WF, Lamberts SW: The acute effect of dexamethasone on plasma leptin concentrations and the relationships between fasting leptin, the IGF-I-IGFBP system, dehydroepiandrosterone, androstenedione and testosterone in an elderly population. Clin Endocrinol 1998;48:621–626.

45 Larsson H, Ahren B: Short-term dexamethasone treatment increases plasma leptin independently of changes in insulin sensitivity in healthy women. J Clin Endocrinol Metab 1996;81:4428–4432.

46 Torpy DJ, Bornstein SR, Cizza G, Chrousos GP: The effects of glucocorticoids on leptin concentrations in humans may be restricted to acute pharmacological dosing. J Clin Endocrinol Metab 1998;83:1821–1822.

47 Tataranni P, Pratley R, Maffei M, Ravussin E: Acute and prolonged administration of glucocorticoids (methylprednisolone) does not affect plasma leptin concentration in humans. Int J Obes 1997;21:327–330.

48 Putignano P, Brunani A, Dubini A, Bertolini M, Pasquali R, Cavagnini F: Effect of small doses of dexamethasone on plasma leptin levels in normal and obese subjects: a dose-response study. J Endocrinol Invest 2003;26:111–116.

49 Rosenbaum M, Pietrobelli A, Vasselli JR, Heymsfield SB, Leibel RL: Sexual dimorphism in circulating leptin concentrations is not accounted for by differences in adipose tissue distribution. Intern J Obes 2001;25:1365–1371.

50 Van Harmelen V, Reynisdottir S, Eriksson P, Thorne A, Hoffstedt J, Lonqvist F, Arner P: Leptin secretion and from subcutaneous and visceral adipose tissue in women. Diabetes 1998;47:913–917.

51 Elbers JMH, Asscheman H, Seidell JC, Frölich M, Meinders AE, Gooren LJG: Reversal of the sex difference in serum leptin levels upon cross-sex hormone administration on transsexuals. J Clin Endocrinol Metab 1997;82:3267–3270.

52 Cohen P, Yang G, Xu X, Soukas AA, Wolfish CS, Friedman JM: Induction of leptin receptor expression in the liver by leptin and food deprivation. J Biol Chem 2005;280:10034–10039.

53 Chan JL, Bluher S, Yiannakouris N, Suchard MA, Kratzsch J, Mantzoros CS: Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids and leptin. Diabetes 2002;51:2105–2112.

54 Misra M, Miller KK, Almazan C, Ramaswamy K, Aggarwal A, Herzog DB, Neubauer G, Breu J, Klibanski A: Hormonal and body composition predictors of soluble leptin receptor, leptin, and free leptin index in adolescent girls with anorexia nervosa and controls and relation to insulin sensitivity. J Clin Endocrinol Metab 2004;89:3486–3495.

55 Gavrila A, Peng CK, Chan L, Mietus JE, Goldberger AL, Mantzoros CS: Diurnal and ultradian dynamics of serum adiponectin in healthy men: comparison with leptin, circulating soluble leptin receptor, and cortisol patterns. J Clin Endocrinol Metab 2003;88:2838–2843.

56 Leal-Cerro A, Considine RV, Peino R, Venegas E, Astorga R, Casanueva FF, Dieguez C: Serum immunoreactive-leptin levels are increased in patients with Cushing's syndrome. Horm Metab Res 1996;28:711–713.

57 Masuzaki H, Ogawa Y, Hosoda K, Miyawaki T, Hanakoa I, Yasuno A, Nishimura H, Yoshimasa Y, Nishi S, Nakao K: Glucocorticoid regulation of leptin synthesis and secretion in humans: elevated plasma leptin levels in Cushing's syndrome. J Clin Endocrinol Metab 1997;82:2542–2547.

58 Cizza G, Lotsikas AJ, Licinio J, Gold PW, Chrousos GP: Plasma leptin levels do not change in patients with Cushing's disease shortly after correction of hypercorticolism. J Clin Endocrinol Metab 1997;82:2747–2750.

59 Weise M, Abad V, Considine RV, Nieman L, Rother KI: Leptin secretion in Cushing's syndrome: preservation of diurnal rhythm and absent response to corticotrophin-releasing hormone. J Clin Endocrinol Metab 1999;84:2075–2079.

60 Widjaja A, Schurmeyer TH, Von zur Muhlen A, Brabant G: Determinants of serum leptin levels in Cushing's syndrome. J Clin Endocrinol Metab 1998;83:600–603.

61 Veldman RG, Frolich M, Pincus SM, Veldhius JD, Roelfsema F: Hyperleptinemia in women with Cushing's disease is driven by high-amplitude pulsatile, but orderly and eurhythmic, leptin secretion. Eur J Endocrinol 2001;144:21–27.

62 Grottoli S, Gauna C, Tassone F, Aimaretti G, Corneli G, Wu Z, Strasburger CJ, Diegeuez C, Casanueva FF, Ghigo E, Maccario M: Both fasting-induced leptin reduction and GH increase are blunted in Cushing's syndrome and in simple obesity. Clin Endocrinol 2003;58:220–228.

63 Kresk M, Silha JV, Jezkova J, Hana V, Marek J, Weiss V, Stepan JJ, Murphy LJ: Adipokine levels in Cushing's syndrome; elevated resistin levels in female patients with Cushing's syndrome. Clin Endocrinol 2004;60:350–357.

64 Van Dielen FMH, Van Veer C, Buurman WA, Greve JWM: Leptin and soluble leptin receptor levels in obese and weight-losing individuals. J Clin Endocrinol Metab 2002;87:1708–1716.

65 Boden G, Chen X, Kolaczynski JW, Polansky M: Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. J Clin Invest 1997;100:1107–1113.

66 Carantoni M, Abbasi F, Azhar S, Schaaf P, Reaven GM: Can changes in plasma insulin concentration explain the variability in leptin response to weight loss in obese women with normal glucose tolerance? J Clin Endocrinol Metab 1999;84:869–872.

67 Havel PJ, Kasim-Karakas S, Mueller W, Johnson PR, Gingerich RL, Stern JS: Relationship of plasma leptin to plasma insulin and adiposity in normal weight and overweight women: effects of dietary fat content and sustained weight loss. J Clin Endocrinol Metab 1996;81:4406–4413.

68 Ueland T, Kristo C, Godang K, Aukrust P, Bollerslev J: Interleukin-1 receptor antagonist is associated with fat distribution in endogenous Cushing's syndrome: a longitudinal study. J Clin Endocrinol Metab 2003;88:1492–1496.

69 Isosaki O, Tsushima T, Miyakawa M, Nozoe Y, Demura H, Seki H: Growth hormone directly inhibits leptin gene expression in visceral fat tissue in fatty Zucker rats. J Endocrinol 1999;161:511–516.

70 Fisker S, Vahl N, Hansen TB, Jorgensen JOL, Hagen C, Orskow H, Christiansen, JS: Serum leptin is increased in growth hormone-deficient adults: relationship to body composition and effects of placebo-controlled growth hormone therapy for one year. Metabolism 1996;46:812–817.

71 Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte D Jr, Woods SC, Seeley RJ, Weigle DS: Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 1996;45:531–535.

72 Motoshima H, Wu X, Sinha MK, Hardy VE, Rosato EL, Barbot DJ, Rosato FE, Goldstein BJ: Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes; effects of insulin and rosiglitazone. J Clin Endocrinol Metab 2002;87:5662–5667.

73 Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE: Structure-function studies of the adipocytes-secreted hormone Acrp30/adiponectin. Implications for metabolic regulation and bioactivity. J Biol Chem 2003;278:9073–9085.

74 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudio K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with body lipoatrophy and obesity. Nat Med 2001;7:941–946.

75 Basu R, Pajvani UB, Rizza RA, Scherer PE: Selective downregulation of the high molecular weight form of adiponectin in hyperinsulinemia and in type 2 diabetes: differential regulation from nondiabetic subjects. Diabetes 2007;56:2174–2177.

76 Hara K, Horikoshi M, Yamauchi T, Yago Y, Miyazaki O, Ebinuma H, Imai Y, Nagai R, Kadowaki T: Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 2009;29:1357–1362.

77 Ouchi N, Kihara S, Arita Y, Nishida M, Matsuyama A, Okamoto Y, Ishigami M, Kuriyama H, Kishida H, Nishizawa H, Hotta K, Muraguchi M, Ohmoto Y, Yamashita S, Funahashi T, Matsuzawa Y: Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation 2001;103:1057–1063.

78 Tan KCB, Xu A, Chow WS, Lam MCW, Ai VHG, Tam SG, Lam KLS: Hypoadiponectinemia is associated with impaired endothelium-dependent vasodilation. J Clin Endocrinol Metab 2004;89:765–769.

79 Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R: Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. Biochem Biophys Res Commun 2002;290:1084–1089.

80 Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB: Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004;291:1730–1737.

81 Makimura H, Mizuno TM, Bergen H, Mobbs CV: Adiponectin is stimulated by adrenalectomy in ob/ob mice and is highly correlated with resistin mRNA. Am J Physiol Endocrinol Metab 2002;283:E1266–E1271.

82 Morton NM, Seckl JR: 11β-Hydroxysteroid dehydrogenase type 1 and obesity. Front Horm Res 2008;36:146–164.

83 Kershaw EE, Morton NM, Dhillon H, Ramage L, Seckl JR, Flier JS: Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity. Diabetes 2005;54:1023–1031.

84 Patel JV, Cummings DE, Girod JP, Mascarenhas AV, Hughes EA, Gupta M, Lip GY, Reddy S, Brotman DJ: Role of metabolically active hormones in the insulin-resistance associated with short-term glucocorticoid treatment. J Negat Results Med 2006;5:14–19.

85 Lewandowski KC, Szosland K, Lewinski A: Short-term dexamethasone administration does not alter serum adiponectin or resistin concentrations in overweight and obese subjects despite an increase in insulin resistance. Clin Endocrinol 2005;62:30–36.

86 Rieth N, Jollin L, Le Panse B, Lecoq AM, Arlettaz A, De Ceaurriz J, Collomp K: Effects of short-term corticoid ingestion on food intake and adipokines in healthy recreationally trained men. Eur J Appl Physiol 2009;105:309–313.

87 Fallo F, Scarda A, Sonino N, Paoletta A, Boscaro M, Pagano C, Federspil G, Vettor R: Effect of glucocorticoids on adiponectin: a study in healthy subjects and in Cushing's syndrome. Eur J Endocrinol 2004;150:339–344.

88 Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G: Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-α expression. Diabetes 2003;52:1779–1785.

89 Libè R, Morpugo PS, Cappiello V, Maffini A, Bondioni S, Locatelli M, Zavanone M, Beck-Peccoz P, Spada A: Ghrelin and adiponectin in patients with Cushing's disease before and after successful transsphenoidal surgery. Clin Endocrinol 2005;62:30–36.

90 Barahona MJ, Sucunza N, Resmini E, Fernandez-Real JM, Ricart W, Moreno-Navarrete JM, Puig T, Farrerons J, Webb SM: Persistent body fat mass and inflammatory marker increase after long-term cure of Cushing's syndrome. J Clin Endocrinol Metab 2009;94:3365–3371.

91 Fukuda I, Hizuka H, Ishikawa Y, Itoh E, Yasumoto K, Murakami Y, Sata A, Tsukada J, Kurimoto M, Okubo Y, Takano K: Serum adiponectin levels in adult growth hormone deficiency and acromegaly. Growth Horm IGF Res 2004;14:449–454.

92 Giavoli C, Cappiello V, Corbetta S, Ronchi CL, Morpurgo PS, Ferrante E, Beck-Peccoz P, Spada A: Different effects of short-and long-term recombinant hGH administration on ghrelin and adiponectin levels in GH-deficient adults. Clin Endocrinol 2004;61:81–87.

93 Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA: The hormone resistin links obesity to diabetes. Nature 2001;409:307–312.

94 Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton CD, Macphee CH, Smith SA: Resistin is expressed in human macrophages and directly regulates by PPARγ activators. Biochem Biophys Res Commun 2003;300:472–476.

95 Ukkola O: Resistin – a mediator of obesity-associated insulin resistance or an innocent bystander? Eur J Endocrinol 2002;147:571–574.

96 Calabro P, Samudio I, Willerson JT, Yeh ET: Resistin promotes smooth muscle cell proliferation through activation of extracellular signal-regulated kinase 1/2 and phosphatidylinositol 3-kinase pathways. Circulation 2004;110:3335–3340.

97 Kawanami D, Maemura K, Takeda N, Harada T, Nojiri T, Imai Y, Manabe I, Utsonomiya K, Nagai R: Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine-endothelial cell interactions. Biochem Biophys Res Commun 2004;314:415–419.

98 Meilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ: Resistin is an inflammatory marker of atherosclerosis in humans. Circulation 2005;111:932–939.

99 Windham BG, Griswold ME, Farasat SM, Ling SM, Carlson O, Egan JM, Ferrucci L, Najjar SS: Influence of leptin, adiponectin, and resistin on the association between abdominal adiposity and arterial stiffness. Am J Hypertens 2010;23:501–507.

100 McManus DD, Lyass A, Ingelsson E, Massaro JM, Meigs JB, Aragam J, Benjamin EJ, Vasan RS: Relations of circulating resistin and adiponectin and cardiac structure and function: The Framingham Offspring Study. Obesity (Silver Spring) 2011 (E-pub ahead of print).

101 Frankel DS, Vesan RS, D'Agostino RB, Benjamin EJ, Levy D, Wang TJ, Meigs JB: Resistin, adiponectin, and risk of heart failure: The Framingham Offspring Study. J Am Coll Cardiol 2009;53:754–762.

102 Weikert C, Westphal S, Berger K, Dierkes J, Möhlig M, Spranger J, Rimm EB, Willich SN, Boeing H, Pischon T: Plasma resistin levels and risk of myocardial infarction and ischemic stroke. J Clin Endocrinol Metab 2008;93:2647–2653.

103 Rajpathak SN, Kaplan RC, Wassertheil-Smoller S, Cushman M, Rohan TE, McGinn AP, Wang T, Strickler HD, Scherer PE, Mackey R, Curb D, Ho GY: Resistin, but not adiponectin and leptin, is associated with the risk of ischemic stroke among postmenopausal women: results from the Women's Health Initiative. Stroke 2011 (Epub ahead of print).

104 Shojima N, Sakoda H, Ogihara T, Fujshiro M, Katagiri H, Anai M, Onishi Y, Ono H, Inukai K, Abe M, Fukushima Y, Kikuchi M, Oka Y, Asano T: Humoral regulation of resistin expression in 3T3-L1 and mouse adipose cells. Diabetes 2002;51:1737–1744.

105 Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R: Tumor necrosis factor-α is a negative regulator of resistin gene expression and secretion in 3T3-L1 adipocytes. Biochem Biophys Res Commun 2001;9:1027–1031.

106 Brown R, Wiesner G, Ur E, Wilkinson M: Pituitary resistin gene expression is upregulated in vitro and in vivo by dexamethasone but is unaffected by rosiglitazone. Neuroendocrinology 2005;81:41–48.

107 Nogueiras R, Gallego R, Gualillo O, Caminos JE, Garcia-Caballero T, Casanueva FF, Dieguez C: Resistin is expressed in different rat tissues and is regulated in a tissue- and gender-specific manner. FEBS Lett 2003;548:21–27.

108 Ermetici F, Malavazos AE, Corbetta S, Morricone L, Dall'Asta C, Corsi MM, Ambrosi B: Adipokine levels and cardiovascular risk in patients with adrenal incidentaloma. Metabolism 2007;56:686–692.

109 Hana V, Silha JV, Justova V, Lacinova Z, Stepan JJ, Murphy LJ: The effects of GH replacement in adult GH-deficient patients: changes in body composition without concomitant changes in the adipokines and insulin resistance. Clin Endocrinol 2004;60:442–450.

110 Fasshauer M, Klein J, Krahlisch S, Lossner U, Klier M, Bluher M, Paschke R: GH is a positive regulator of tumor necrosis factor-α-induced adipose related protein in 3T3-L1 adipocytes. J Endocrinol 2003;178:523–531.

111 Hotamisligil G, Arner P, Caro J, Atkinson R, Spiegelman B: Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 1995;95:2409–2415.

112 Yudkin JS, Eringa E, Stehouwer CD: 'Vasocrine' signaling from perivascular fat: a mechanism linking insulin resistance to vascular disease. Lancet 2005;365:1817–1820.

113 Ross R: Atherosclerosis: an inflammatory disease. N Engl J Med 1999;340:115–126.

114 Cesari M, Penninx BW, Newman AB, Kritchevsky SB, Nicklas BJ, Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, Pahor B: Inflammatory markers and onset of cardiovascular events: results from the Health ABC Study. Circulation 2003;108:2317–2322.

115 Ridker PM, Rifai N, Pfeifer M, Sacks F, Lepage S, Braunwald E: Elevation of tumor necrosis factor-α and increased risk of recurrent coronary events after myocardial infarction. Circulation 2000;101:2149–2153.

116 Swain MG, Appleyard CB, Wallace JL, Maric M: TNF-α facilitates inflammation-induced glucocorticoid secretion in rats with biliary obstructions. J Hepatol 1997;26:361–368.

117 Artz E, Kovalovsky D, Igaz LM, Costas M, Plazas P, Refojo D, Paez Pereda M, Reul JM, Stalla G, Holsboer F: Functional cross-talk among cytokines, T-cell receptor, and glucocorticoid receptor transcriptional activity and action. Ann N Y Acad Sci 2009;917:672–677.

118 Bernardini R, Kamilaris TC, Calogero AE, Johnson EO, Gomez MT, Gold PW, Chrousos GP: Interactions between tumor necrosis factor-α, hypothalamic corticotrophin-releasing hormone, and adrenocorticotropin secretion in the rat. Endocrinology 1990;126:2876–2881.

119 Gaillard RC, Turnill D, Sappino P, Muller AF: Tumor necrosis factor-α inhibits the hormonal response of the pituitary gland to hypothalamic releasing factors. Endocrinology 1990;127:101–106.

120 Judd AM, Call GB, Barney M, McImoil CJ, Balls AG, Adams A, Oliveira GK: Possible function of IL-6 and TNF as intra-adrenal factors in the regulation of adrenal steroid secretion. Ann N Y Acad Sci 2000;917:628–637.

121 Bujalska LJ, Kumar S, Stewart PM: Does central obesity reflect 'Cushing's disease of the omentum'? Lancet 1997;349:1210–1213.

122 Sewter CP, Digby JE, Blows F, Prins J, O'Rahilly S: Regulation of tumour necrosis factor-α release from human adipose tissue in vitro. J Endocrinol 1999:163:33–38.

123 Zilberfarb V, Siquier Q, Strosberg AD, Issad T: Effect of dexamethasone on adipocyte differentiation markers and tumor necrosis factor-α expression in human PAZ6 cells. Diabetologia 2003;44:377–386.

124 Elenkov IJ, Chrousos GP: Stress hormones, pro-inflammatory and anti-inflammatory cytokines, and autoimmunity. Ann N Y Acad Sci 2002;966:290–303.

125 Zawacki JK, Hunt JL, Gamelli RL, Filkins JP: Glucocorticoid regulation of hepatic TNF production following cecal ligation and puncture sepsis. Shock 1997;8:141–145.

126 Patel JV, Cummings DE, Girod JP, Mascarenhas AV, Hughes EA, Gupta M, Lip GY, Reddy S, Brotman DJ: Role of metabolically active hormones in the insulin resistance associated with short-term glucocorticoid treatment. J Negat Results Biomed 2006;11:5–14.

127 DeRijk R, Michelson D, Karp B, Petrides J, Galliven E, Deuster P, Paciotti G, Gold PW, Sternberg EM: Exercise and circadian rhythm-induced variations in plasma cortisol differentially regulate interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) production in humans: high sensitivity of TNF-α and resistance of IL-6. J Clin Endocrinol Metab 1997;82:2182–2191.

128 Russell M, Bredella M, Tsai P, Mendes N, Miller KK, Klibanski A, Misra M: Relative growth hormone deficiency associated with increased cardiovascular risk markers in obese adolescent girls. J Clin Endocrinol Metab 2009;94:2864–2871.

129 Papanicolau DA, Tsigos C, Oldfield EH, Chrousos GP: Acute glucocorticoid deficiency is associated with plasma elevations of interleukin-6: does the latter participate in the symptomatology of the steroid withdrawal syndrome and adrenal insufficiency? J Clin Endocrinol Metab 1996;81:2303–2306.

130 Kristo C, Godang K, Ueland T, Lien E, Aukrust P, Froland SS, Bollerslev J: Raised serum levels of interleukin-8 and interleukin-18 in relation to bone metabolism in endogenous Cushing's syndrome. Eur J Endocrinol 2002;146:389–395.

131 Merola B, Longobardi S, Colao A, Di Somma C, Ferone D, Di Rella F, Pivonello R, Covelli V, Annunziato L, Lombardi G: Tumor necrosis factor-α increases after corticotropin-releasing hormone administration in Cushing's disease. In vivo and in vitro studies. Neuroendocrinology 1996;64:393–397.

132 Chrousos GP: The hypothalamic-pituitary adrenal axis and the immune-mediated inflammation. N Engl J Med 1995;332:1351–1362.

133 Arikan S, Bahceci M, Tuzcu A, Gokalp D: Serum tumor necrosis factor-α and interleukin-8 levels in acromegalic patients: acromegaly may be associated with moderate inflammation. Clin Endocrinol 2009;70:498–501.

134 Serri O, St-Jacques P, Sartippour M, Renier G: Alteration of monocyte function in patients with GH deficiency: effect of substitutive GH therapy. J Clin Endocrinol Metab 1999;84:58–63.

135 Mohamed-Ali V, Pinkney JK: Adipose tissue as an andocrine and paracrine organ. Int J Obes Relat Metab Disord 1998;22:1145–1158.

136 Vozarova B, Weyer C, Hanson K, Tataranni PA, Bogardus C, Pratley RE: Circulating IL-6 in relation to adiposity, insulin action and insulin secretion. Obes Res 2001;9:414–417.

137 Fasshauer M, Paschke R: Regulation of adipocytokines and insulin resistance. Diabetologia 2003;46:1594–1603.

138 Bastard JP, Jardel C, Bruckett E, Blondy P, Capeau J, Laville M, Vidal H, Hainque B: Elevated levels of interleukin-6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 2000;85:3338–3342.

139 Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V: Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 1999;148:209–214.

140 Harris TB, Ferrucci L, Tracy RP, Corti MC, Wacholder S, Ettinger WH Jr, Heimowitz H, Cohen HJ, Wallace R: Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am J Med 1999;106:506–512.

141 Liuzzo G, Baisucci LM, Gallimore JR, Caligiuri G, Buffon A, Rebuzzi AG, Pepys MB, Maseri A: Enhanced inflammatory response in patients with preinfarction unstable angina. J Am Coll Cardiol 1999;34:1696–1703.

142 Turnbull AV, Rivier CL: Regulation of the hypothalamic-pituitary adrenal axis by cytokines: actions and mechanisms of action. Physiol Rev 1999;79:1–71.

143 Bethin KE, Vogt SK, Muglia LJ: Interleukin-6 is an essential, corticotropin-releasing hormone-independent stimulator of the adrenal axis during immune system activation. Proc Natl Acad Sci USA 2006;97:9317–9322.

144 Salas MA, Evans SW, Levell MJ, Whicher JT: Interleukin-6 and ACTH act synergistically to stimulate the release of corticosterone from adrenal gland cells. Clin Exp Immunol 1990;79:470–473.

145 Pereda MP, Lohrer P, Kovalovsky D, Perez Castro C, Goldberg V, Losa M, Chervin A, Berner S, Molina H, Stalla GK, Renner U, Artz E: Interleukin-6 is inhibited by glucocorticoids and stimulates ACTH secretion and POMC expression in human corticotroph pituitary adenomas. Exp Clin Endocrinol Diabetes 2000;108:202–207.

146 Sarlis NJ, Stephanou A, Knight RA, Lightman SL, Chowdrey HS: Effects of glucocorticoids and chronic inflammatory stress upon anterior pituitary interleukin-6 mRNA expression in the rat. Br J Rheumatol 1993;32:653–657.

147 Fried SK, Bunkin DA, Greenberg AS: Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoids. J Clin Endocrinol Metab 1998;83:847–850.

148 Papanicolau DA, Petrides JS, Tsigos C, Bina S, Kalogeras KT, Wilder R, Gold PW, Deuster PA, Chrousos GP: Exercise stimulates interleukin-6 secretion: inhibition by glucocorticoids and correlation with catecholamines. Am J Physiol 1996;271:E601–E605.

149 Mastorakos G, Chrousos GP, Weber JS: Recombinant interleukin-6 activates the hypothalamic-pituitary-adrenal axis in humans. J Clin Endocrinol Metab 1993;77:1690–1694.

150 Kushlinskii NE, Britvin TA, Kazantseva IA, Baronin AA, Polikarpova SB, Bogatyrev OP, Tishenina RS, Kalinin AP: Serum interleukin-6 in patients with adrenal tumors. Bull Exp Biol Med 2004;137:273–275.

151 Umemura S, Nyui N, Tamura K, Hibi K, Yamaguchi S, Nakamuru M, Ishigami T, Yabana M, Kihara M, Inoue S, Ishii M: Plasma angiotensinogen concentrations in obese patients. Am J Hypertens 1997;10:629–633.

152 Van Harmelen V, Elizalde M, Ariapart P, Bergstedt-Lindqvist S, Reynisdottir S, Hoffstedt J, Lundkvist I, Bringman S, Arner P: The association of human adipose angiotensinogen gene expression with abdominal fat distribution in obesity. Int J Obes Relat Metab Disord 2000;24:673–678.

153 Kim S, Solatni-Bejnood M, Quignard-Boulange A, Massiera F, Teboul M, Ailhaud G, Kim JH, Moustaid-Moussa N, Voy BH: The adipose renin-angiotensin system modulates systemic markers of insulin sensitivity and activates the intrarenal renin-angiotensin system. J Biomed Biotech 2006;2006:27012.

154 Massiera F, Bloch-Faure M, Ceiler D, Murakami K, Fukamizu A, Gasc JM, Quignard-Boulange A, Negrel R, Ailhaud G, Seydoux J, Meneton P, Teboul M: Adipose angiotensinogen is involved in adipose tissue growth and blood pressure regulation. FASEB J 2001;15:2727–2729.

155 Aubert J, Darimont C, Safonova I, Ailhaud G, Negrel R: Regulation by glucocorticoids of angiotensinogen gene expression and secretion in adipose cells, Biochem J 1997;328:701–706.

156 Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. Science 2001;294:2166–2170.

157 Saint-Marc P, Kozac LP, Aihaud G, Darimont C, Negrel R: Angiotensin II as a trophic factor of white adipose tissue: stimulation of adipose cell formation. Endocrinology 2001;142:487–492.

158 Gorzelniak K, Engeli S, Janke J, Luft FC, Sharma AM: Hormonal regulation of the human adipose-tissue renin-angiotensin system: relationship to obesity and hypertension. J Hypertens 2002;20:965–973.

159 Engeli S, Bohnke J, Feldpausch M, Gonzelniak K, Heintze U, Janke J, Luft FC, Sharma AM: Regulation of 11β-HSD genes in human adipose tissue: influence of central obesity and weight loss. Obes Res 2004;12:9–17.

160 Haller H, Hensen J, Bahr V, Oelkers W: Effects of angiotensin II infusion on the early morning surge of ACTH and o-CRH-provoked ACTH secretion in normal man. Acta Endocrinol 1986;112:150–156.

161 Calogero AE, Fornito MC, Aliffi A, Vicari E, Moncada ML, Mantero F, Polosa P, D'Agata R: Role of peripherally infused angiotensin II on the human hypothalamic-pituitary-adrenal axis. Clin Endocrinol 1991;34:183–186.

162 Rayyis SS, Horton R: Effect of angiotensin II on adrenal and pituitary function in man. J Clin Endocrinol Metab 1971;32:539–546.

163 Semple PF, Buckingham JC, Mason PA, Fraser R: Suppression of plasma ACTH concentration by angiotensin II infusion in normal humans and in a subject with a steroid 17α-hydroxylase defect. Clin Endocrinol 1979;10:137–144.

164 Sanchez-Lemus E, Benicky J, Pavel J, Saavedra JM: In vivo angiotensin II AT(1) receptor blockade selectively inhibits LPS-induced innate immune response and ACTH release in rat pituitary gland. Brain Behav Immun 2009;296:R1376–R1384.

165 Saruta T, Suzuki H, Handa M, Igarashi Y, Kondo K, Senba S: Multiple factors contribute to the pathogenesis of hypertension in Cushing's syndrome. J Clin Endocrinol Metab 1986;62:275–279.

166 Shibata H, Suzuki H, Maruyama T, Saruta T: Gene expression of angiotensin II receptor in blood cells of Cushing's syndrome. Hypertension 1995;26:1003–1010.

167 Festa A, D'Agostino R Jr, Tracy RP, Haffner SM: Insulin resistance atherosclerosis study. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 2002;51:1131–1137.

168 Juhan-Vague I, Alessi MC, Mavri A, Morange PE: Plasminogen activator inhibitor-1, inflammation, obesity, insulin resistance and vascular risk. J Thromb Haemost 2003;1:1575–1579.

169 Halleux CM, Declerck PJ, Tran SL, Detry R, Brichard SM: Hormonal control of plasminogen activator inhibitor-1 gene expression and production in human adipose tissue: stimulation by glucocorticoids and inhibition by catecholamines. J Clin Endocrinol Metab 1999;84:4097–4105.

170 Ayachi SE, Paulmyer-Lacroix O, Verdier M, Alessi MC, Dutour A, Grino M: 11β-Hydroxysteroid dehydrogenase type 1-driven cortisone reactivation regulates plasminogen activator inhibitor type 1 in adipose tissue of obese women. J Thromb Haemost 2006;4:621–627.

171 Darmon P, Dadoun F, Boullu-Ciocca S, Grino M, Alessi MC, Dutour A: Insulin resistance induced by hydrocortisone is increased in patients with abdominal obesity. Am J Physiol Endocrinol Metab 2006; 291:E995–E1002.

172 Patrassi GM, Sartori MT, Viero ML, Scarano L, Boscaro M, Girolami A: The fibrinolytic potential in patients with Cushing's disease: a clue to their hypercoagulable state. Blood Coag Fibrinolysis 1992;3:789–793.

173 Fatti LM, Bottasso B, Invitti C, Coppola R, Cavagnini F, Mannucci PM: Markers of activation of coagulation and fibrinolysis in patients with Cushing's syndrome. J Endocrinol Invest 2000;23:145–150.

174 Sartorio A, Cattaneo M, Bucciarelli P, Bottasso B, Porretti S, Epaminonda P, Faglia G, Arosio M: Alterations of haemostatic and fibrinolytic markers in adult patients with growth hormone deficiency and with acromegaly. Exp Clin Endocrinol Diabetes 2000;108:486–492.

175 Devin JK, Blevins LS, Verity DK, Chen Q, Bloodworth JR, Jovington J, Vaughan DE: Markedly impaired fibrinolytic balance contributes to cardiovascular risk in adults with growth hormone deficiency. J Clin Endocrinol Metab 2007;92:3633–3639.

176 Van der Lely AJ, Tschop M, Heiman ML, Ghigo E: Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. Endocr Rev 2004;25:426–457.

177 Broglio F, Gottero C, Prodam F, Gauna C, Muccioli G, Papotti B, Abribat T, Van der Lely AJ, Ghigo E: Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans. J Clin Endocrinol Metab 2004; 89:3062–3065.

178 Soeki T, Kishimoto I, Schwenke DO, Tokudome T, Horio T, Yoshida M, Hosoda H, Kangawa K: Ghrelin suppresses cardiac sympathetic activity and prevents early left ventricular remodeling in rats with myocardial infarction. Am J Physiol Heart Circ Physiol 2007;294:H426–H432.

179 Li WG, Gavrila D, Liu X, Wang L, Gunnlaugsson S, Stoll LL, McCormick ML, Sigmund CD, Tang C, Weintraub NL: Ghrelin inhibits proinflammatory responses and nuclear factor-κB activation in human endothelial cells. Circulation 2004;109:2221–2226.

180 Tesauro M, Schinzari F, Iantorno M, Rizza S, Melina D, Lauro D, Cardillo C: Ghrelin improves endothelial function in patients with metabolic syndrome. Circulation 2005;112:2986–2992.

181 Vestergaard ET, Andersen NH, Hansen TK, Rasmussen LM, Moller N, Sorensen KE, Sloth E, Jorgensen JO: Cardiovascular effects of intravenous ghrelin infusion in healthy young men. Am J Physiol Heart Circ Physiol 2007;293:H3020–H3026.

182 Okumura H, Nagaya N, Enomoto M, Nakagawa E, Oya H, Kangawa K: Vasodilatory effect of ghrelin, an endogenous peptide from the stomach. J Cardiovac Pharmacol 2002;39:779–783.

183 Copinschi G, Van Onderbengen A, L'Hermite-Baleriaux M, Mendel CM, Caufriez A, Leproult A, Bolognese JA, De Smet M, Thorner MO, Van Cauter E: Effects of a 7-day treatment with a novel, orally active, GH secretagogue, MK-677, on 24-hour GH profiles, insulin-like-growth factor I, and adrenocortical function in normal young men. J Clin Endocrinol Metab 1996;81: 2776–2782.

184 Mozid AM, Tringali G, Forsling ML, Hendricks MS, Ajodha S, Edwards R, Navarra P, Grossman AB, Korbonits M: Ghrelin is released from rat hypothalamic explants and stimulates corticotrophin-releasing hormone and arginine-vasopressin. Horm Metab Res 2003;35:455–459.

185 Hickey GJ, Drisko J, Faidley T, Chang CC, Anderson LL, Nicolich S, McGuire L, Rickes E, Krupa D, Feeney W, Friscino B, Cunningham B, Frazier E, Chen H, Laroque P, Smith RG: Mediation by the central nervous system is critical to the in vivo activity of the GH secretagogue L-692,585. J Endocrinol 1996;148:371–380.

186 Ghigo E, Arvat E, Ramunni J, Colao A, Gianotti L, Deghenghi R, Lombardi G, Camanni F: Adrenocorticotropin- and cortisol-releasing effect of hexarelin, a synthetic growth hormone-releasing peptide, in normal subjects and patients with Cushing's syndrome. J Clin Endocrinol Metab 1997; 82:2439–2444.

187 Korbonits M, Bustin SA, Kojima M, Jordan S, Adams EF, Lowe DG, Kangawa K, Grossman AB: The expression of the growth hormone secretagogue receptor ligand ghrelin in normal and abnormal human pituitary and other neuroendocrine tumors. J Clin Endocrinol Metab 2001;86:881–887.

188 Pecori Giraldi F, Bucciarelli L, Saccani A, Scacchi M, Pesce S, Losa M, Cavagnini F: Ghrelin stimulates adrenocorticotrophic hormone (ACTH) secretion by human ACTH-secreting pituitary adenomas in vitro. J Neuroendocrinol 2007;19:208–212.

189 Ueberberg B, Unger N, Sheu SY, Walz MK, Schmid KW, Saeger W, Mann K, Petersenn S: Differential expression of ghrelin and its receptors (GHS-R1a) in various adrenal tumors and normal adrenal gland. Horm Metab Res 2008;40:181–188.

190 Caminos JE, Nogueiras R, Blanco M, Seoane LM, Bravo S, Alvarez CM, García-Caballero T, Casanueva FF, Diéguez C: Cellular distribution and regulation of ghrelin messenger ribonucleid acid in the rat pituitary gland. Endocrinology 2003;144:5089–5097.

191 Otto B, Tschop M, Heldwein W, Pfeiffer AFH, Diederich S: Endogenous and exogenous glucocorticoids decrease plasma ghrelin in humans. Eur J Endocrinol 2004; 151:113–117.

192 Giordano R, Picu A, Pagotto U, De Iasio R, Bonelli L, Prodam F, Broglio F, Marafetti L, Pasquali R, Maccario M, Ghigo E, Arvat E: The negative association between total ghrelin levels, body mass and insulin secretion is lost in hypercortisolemic patients with Cushing's disease. Eur J Endocrinol 2005;153:535–543.

193 McLaughlin T, Abbasi F, Lamendola C, Frayo RS, Cummings DE: Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. J Clin Endocrinol Metab 2004;89:1630–1635.

194 Janssen JAM, van der Toorn FM, Hofland LJ, van Koetsveld P, Broglio F, Ghigo E, Lamberts SW, Van der Lely A: Systemic ghrelin levels in subjects with growth hormone deficiency are not modified by one year of growth hormone replacement therapy. Eur J Endocrinol 2001;145:711–716.

195 Jarkovska Z, Rosicka M, Marek J, Hana V, Weiss V, Justova V, Lacinova Z, Haluzik M, Haas T, Kresk M: Plasma levels of total and active ghrelin in acromegaly and growth hormone deficiency. Physiol Res 2006;55: 175–181.

196 Ryber L, Obrink K, Houe N, Frystyk J, Jorgensen JO: Serum ghrelin levels are suppressed in hypopituitary patients following insulin-induced hypoglycaemia irrespective of GH status. Clin Endocrinol 2006;65: 210–214.